THE EFFECTS OF INBREEDING AND CROSSING ON LITTER SIZE IN MICE

by

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Submitted to the University of Edinburgh as a Thesis in fulfilment of the requirements for the degree of Doctor of Philosophy.

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October, 1956.

CONTENTS

I. INTRODUCTION

"Appreciation of the practical value of hybrid vigour is as old as the mule, but its scientific investigation began only relatively recently." These words were spoken by Professor Mather only a year or so ago. Hybrid vigour is the basis of the commercial production of maize and certain other agricultural crops in the United States; hybrid maize, in particular, has assumed the proportions of a major industry. This breeding practice has by now been extended to the production of hybrid chicks and, to a lesser extent, hybrid pigs. For all this, the factors actually exploited seem to be but little understood.

"Hybrid vigour" is conveniently defined as "an excess vigour of a hybrid over the average vigour of its parents (Richey, 1946). The same phenomenon is often referred to as "heterosis", a contraction of the word heterozygosis, and originally proposed by Shull in 1914 simply as a synonym for "hybrid vigour" (Shull, 1948). Some workers seem to restrict the use of the term "hybrid vigour" to the vigour of crosses between inbred lines; in such cases, "heterosis" is a wider term denoting vigour resulting from a general increase in heterozygosity. Dobzhansky (1952) distinguishes between heterosis in characters directly related to fitness ("euheterosis" in Dobzhansky's terminology) and "luxuriance", - vigour resulting in an organism being larger or faster growing or otherwise exceeding the parental forms in some quality that does not necessarily result in increased fitness. In practice, however, this distinction may not be an easy one to make, and from the plant or animal breeders' point of view, "luxuriance" is often the desired objective. In what follows, the term "hybrid vigour" will be used throughout, as it seems to be a more descriptive and a better defined term than any of its alternatives.

It has long been recognised that hybrid vigour and inbreeding depression are complementary facets of the same phenomenon. This was realised by Shull as far back

as 1908. Any explanation of the one will therefore automatically account for the other, and any comprehensive investigation of the field will involve the study of both.

The rediscovery of Mendel's work at the beginning of this century gave the phenomenon of hybrid vigour a theoretical interest. It was natural that an explanation should be sought on genetical lines. Yet, some of the first suggestions were, strictly speaking, of a non-Mendelian kind. Shull (1908, 1911) and East (1908) believed that there exists a stimulus on crossing due to the genetic difference in the germ plasms, and that the stimulus was proportional to the amount of the difference. This represented little, if any, advance on the views of Darwin (1876) who attributed the extra vigour to the "sexual elements (being) in some degree differentiated" (quoted by Mather, 1955).

However, a rather different hypothesis was soon to be proposed. Keeble and Pellew (1910) found the hybrid between two varieties of the garden pea to be taller than either parent. These hybrids, when selfed, gave rise in the F_2 to four distinct types with respect to height - the F_1 type, types resembling the two parental varieties, and a dwarf. From their data, Keeble and Pellew were able to postulate two dominant genes affecting height - one gene giving longer internodes and the other a thicker stem. Both dominants were acting together in the hybrid to give the increase in height. Further, they remark - "The suggestion may be hazarded that the greater height and vigour which the F_1 generation of hybrids commonly exhibit may be due to the meeting in the zygote of dominant growth factors of more than one allelomorphic pair, one (or more) provided by gametes of one parent, the other (or others) by the gametes of the other parent". This is the essence of what is known as the "Dominance Theory" to explain hybrid vigour.

In the same year, Bruce presented a theoretical but more general exposition of the same hypothesis. He showed algebraically that a hybrid population will contain fewer

homozygous recessives at a particular locus than the mean of two parent populations with unequal gene frequencies. The dominance theory of course depends on the observation, first noted by Davenport (1908), that dominance is usually associated with beneficial effects and recessiveness with detrimental effects.

There were early objections to the dominance theory on two grounds. Firstly, it should be possible to select pure-breeding lines containing all the dominants and therefore equal in vigour to the hybrids; this had not proved to be at all possible. Secondly, the F_2 generation should have a skewed distribution, many individuals being equal to the F_1, the others showing a progression of reduction in vigour; again this did not agree with observation. The first of these objections was removed by Jones (1917). By taking into account Morgan's theory of linkage, Jones considered that a multifactorially determined character would probably be controlled by genes on several chromosomes, and that furthermore, linkage of favourable dominants with harmful recessives might not readily break up. Under these conditions, the accumulation of all homozygous dominant loci in any one individual becomes very improbable. Jones showed also that linkage would explain the absence of skewness in the F_2 generation. This latter point is further elaborated by Collins (1921) who showed that even in the absence of linkage, the skew distribution of the F_2 generation due to dominance becomes less marked as the number of factors controlling the character is increased.

East (1936) points out that Jones' "Dominance of Linked Factors Hypothesis", as it became to be called, appeared to be so probable that even in the absence of any direct proof, it remained unchallenged at least until 1930. It seems therefore that simple dominance remained the only widely accepted genetic explanation of hybrid vigour throughout this time.

In the middle thirties, however, there emerged an alternative hypothesis that was in a sense a return to ideas similar to the original ones of Shull and East. It was that heterozygosity itself produces an increase in vigour. In genetic terms, this requires that the heterozygote at a particular locus is superior to either homozygote.

This was first explicitly expounded by East (1936), though he seems to have chosen an extreme version which did not become generally acceptable. East postulated that loci with a series of non-defective allelomorphs were a common occurrence. The action of each allele was to some extent cumulative, the cumulative action being progressively greater the more divergent the alleles were in function. The underlying concept of heterozygote superiority was subsequently favoured by Singleton (1943), Jones (1944, 1945) and particularly by Hull (1945). Hull suggested the word "overdominance" to describe this form of allelic interaction, and the term has come into common use. Hull's basic argument for overdominance was a simple one; it was that his maize hybrids often exceeded in yield the sum of the two parent inbreds; this is not possible on a simple dominance scheme. The validity of this argument is of course entirely dependent on the absence of epistasis. Evidence on this point quoted by Crow (1952) is conflicting, though it all comes from maize data. Much more convincing is a paper by Jinks (1955), who analysed data from a variety of plant material, and whose method of analysis clearly distinguishes between the interaction of alleles at the one locus and interaction between non-allelic genes. Jinks states that wherever he found apparent overdominance, he also found non-allelic interaction; that the removal of non-allelic interaction always reduced the degree of overdominance; and that in the one case where the non-allelic interaction could be removed entirely, it led to the complete disappearance of the spurious overdominance. Jinks' work therefore seems to cast serious doubt on Hull's basic premise that epistasis is unimportant.

The latest and perhaps, by now, the best-known exposition of the importance of heterozygosity per se is of course that of Lerner (1954). Lerner considers that, in cross-breeding organisms, multiple heterozygotes have a higher selective advantage than homozygotes, in that natural selection favours intermediate rather than extreme phenotypes. Evolution has established levels of obligate heterozygosity in natural populations. To do justice with Dr. Lerner's thesis would naturally require a very much more comprehensive treatment than this, but it can be seen how hybrid vigour would

be readily explicable along these lines.

We see therefore that historically, favourite explanations of the phenomenon of hybrid vigour seem to have alternated between two theories that differ essentially in nothing more than the degree of dominance concerned. One theory postulates that in hybrids, deleterious recessive genes are masked by their superior alleles, and is based on the observed correlation between dominance and beneficial effects. The other theory implicates hybridity per se as the causative factor; this requires that, at a particular locus, the heterozygote is superior to either homozygote, i.e. overdominance is operative. Both theories were originally proposed in the early years of this century and reasserted with little modification from time to time. Both are still current to-day. In practice, both hypotheses often lead to the same expectation (see, for instance, Robinson et al., 1956; Crow, 1952). With close linkage, dominant alleles in repulsion would be indistinguishable in their effect from a single overdominant locus.

The dominance theory is particularly plausible. Wild populations, whenever they are examined, reveal many harmful recessives and even lethals. One of the most frequently quoted examples is that of Dobzhansky et al. (1942). In a population of Drosophila pseudoobscura which they studied, only 3% of the flies were free from detectable deleterious recessives. These recessives are unavoidably revealed on inbreeding, and any theory of inbreeding and hybrid vigour must therefore take account of dominance, partial or complete.

The overdominance theory is not, at first sight, so compelling. Crow (1952) points out that if only a small proportion of loci are of an overdominant type, these may nevertheless be the major factor in the population variance. But the acceptance of the theory must be based on the unequivocal establishment of individually over-dominant loci. This has proved to be difficult, but a fair number of such claims are to be found in the literature. These are quoted by Crow (1948, 1952), Buzzati-Traverso (1952) and notably by Lerner (1954). However, other explanations may often be suggested, as it is seldom, if ever, possible to rule out interaction between non-allelic genes.

This of course raises the question of how important are these non-allelic interactions. Their possible existence in quantitative characters was first raised by Rasmusson (1934), in a theoretical paper. A fuller treatment is developed by Mather (1943) and, paying particular regard to inbreeding depression and hybrid vigour, by the same author (1955). Thus, to quote Mather (1955) - "In outbreeding species, for example, the naturally occurring genotypes will virtually always be partially heterozygous, and natural selection will therefore favour those combinations which combine in homologous pairs to give a good balance. Combinations will, on the other hand, seldom be exposed in the homozygous condition, so that no selection will have been acting to pick out from the great mass of possible genotypes those which show a good homozygous or internal balance. Inbreeding would thus virtually always lead to a phenotypic depression, reflecting a balance which was poor because it was untested, and vanishing when the tested hybrid balance was restored by crossing." Reference has already been made to the work of Jinks (1955), reporting the common occurrence of non-allelic interaction in a variety of material. Its importance should not therefore be underestimated.

We thus see that current hypotheses of hybrid vigour involve both intra- and inter-locular interactions. It is recognised that the various theories are not necessarily collectively exhaustive. Furthermore, it seems reasonable to expect that they act with different force in different circumstances. The suggestion that any one generally operates to the exclusion of the others would seem, at present, to be unwarranted.

Much of the previous work on inbreeding and hybrid vigour seems to have been motivated by the desire to improve characters of economic importance. To this end, the inbreeding stage is characterised by vigorous selection between the lines, and the crossing programme is designed to discover the best crosses. Many if not most of the possible genotypes are therefore the victims of selection, and on account of this, one important piece of information seems to be entirely lacking. We are unable to

distinguish between the relative importance of the breeding system per se and the selection that seems invariably to accompany it. The successful outcome of an inbreeding and crossing programme is the finding of a cross or crosses that exceed the level of the outbred population from which the lines are derived. But what would the outcome be in the absence of selection? Would inbreeding and crossing alone result in any improvement? Or is the breeding system merely a means of revealing and thereby eliminating undesirable genotypes?

Whatever the true explanation of inbreeding depression and hybrid vigour may be, theoretical considerations would lead us to the following conclusions. If a large number of inbred lines is derived from an outbred population, and these lines are crossed at random without selection, the progeny of any single cross then corresponds to one individual from the original outbreds, that individual being of course replicated in all the progeny of the same cross. Likewise, the F_1 population as a whole corresponds to the parent outbred population. The mean of any metrical trait would therefore have the same expectation in both outbreds and crossbreds, and the best crosses would equal but not surpass the best individuals from the outbred population. On this view, hybrids are endowed with no advantage over outbreds other than that the best individuals can be replicated at will. Where is the mean? Does it agree with these theoretical considerations? This is another way of expressing the questions posed earlier, and the primary aim of the work to be described here was to provide the information, to be obtained by empirical observation. The answer obtained will in no way enable us to discriminate between the theories of hybrid vigour, but it might test their adequacy in aggregate. It might indicate whether any other factor, hitherto unsuspected, is operative. But more important, it will show whether inbreeding and crossing have intrinsic properties of their own, other than affording means of rapid and effective selection. This question seems to merit investigation.

The organism chosen for the work was the mouse, the character litter size. The mouse was chosen because of the desirability of working with a convenient mammal; the

results might then be more readily applicable to farm animals, to pigs in particular. Litter size was chosen because it was known, from previous work in this laboratory and elsewhere, that its depression on inbreeding is marked. In addition, other work on litter size was already in progress, making the comparative study of the results possible.

II. THE CHARACTER - LITTER SIZE

In wild populations, fertility is a major component of fitness. Likewise, the economic value of domestic animals is usually directly related to their fertility. Furthermore, there can be no genetic improvement of a stock unless sufficient progeny are available to provide a choice of parents for the next generation. By any standard therefore, the fertility of an animal is one of its most important characteristics.

In polytocous animals, fertility is governed by two components - litter size, and the frequency with which litters are produced. These do not seem to be causally connected, as Figure 1 shows no obvious relationship, in an outbred unselected stock, between the size of the first litter and the interval between it and the second. Litter size therefore provides a direct reflection of the animal's fertility, as it varies independently of the other component. This was also found by Murray (1934). As healthy animals normally produce litters at regular intervals, litter size is often the major determinant of fertility.

"Litter size" would appear to be a selfexplanatory term - the number born in a litter. Mice unfortunately complicate the issue by disposing of many of their still-born young and neo-natal deaths, and occasionally some others as well. The number of young found is therefore influenced by the time interval between birth and the examination of the litter, litters being often born at night. Cages are examined once daily, the number of live young being recorded as the litter size, though a note is made of any dead young or remains that are seen.

For genetical work, mice have rather a low rate of reproduction, though it compares favourably with other mammals. In view of this, it is very convenient to work where possible with first litters only. The question arises - is this first litter representative of the mouse's fertility? We require to know the repeatability of litter size. Two stocks used previously in this laboratory provided suitable data for correlating the size of the first and second litters. The first of these, EV, has

been described by Falconer and Robertson (1956), and the second stock, Z, by Falconer (1954). These were the figures obtained:-

	Stock	d.f.	Repeatability	Probability level
	EVE	95	0.488	.001
	EVC	94	0.298	.01 .001
Mean	EV	191	0.396	.001
	Z	391	0.405	.001

The higher repeatability obtained in EVE compared to EVC would be readily explained by the fact that in the former, litters were taken from females of extreme body weight (see later for the effect of body weight on litter size). EVC was the control stock. Such an explanation is not required, however, as the difference between the two is not statistically significant, and the figure obtained by pooling the data agrees nicely with that obtained from the Z stock.

This correlation of 0.4 between first and second litters, while being reasonably satisfactory, indicates that for accurate measurement of litter size, more than one litter should be recorded where possible. When a mouse becomes pregnant to a post-partum oestrus, this presents no difficulty, as the information becomes available well before the mice from the first litter are mated. Unfortunately, only a proportion of mice do this, and the collection of sufficient information about second litters to be of material assistance would inordinately prolong the generation interval. Progress per generation must be considered in conjunction with the generation interval, as the important factor is progress per unit of time. It does not seem that with a character such as litter size in the mouse, the consideration of an extra observation is worth while. Lush (1945) gives the following formula:-

Progress under selection per generation, selecting on an average of n records = $\frac{n}{1+(n-1)r}$ times the progress made if selecting on one record per animal, where r is the repeatability of the observation. Substituting our figure of 0.4 for the

repeatability, progress per generation would be increased by 20% by taking note of second litters. This modest advantage would be nullified or even outweighed by the longer generation interval. It was clear therefore at the outset that the work would have to be done on first litters only.

Litter size shows a normal distribution about its mean, or at least a distribution sufficiently close to normality for the usual statistical tests to be valid without transformation of scale. Figure 2 shows the distribution of the size of the first litters in an unselected control stock, JC, which will be referred to later.

Litter size is a complex character, depending upon three major components:-

(i) The number of eggs shed.

(ii) The number of eggs fertilised.

(iii) The number of zygotes carried to term.

As a subsidiary experiment, an analysis was attempted of the effects of inbreeding and crossing on these components separately. They must therefore be considered in greater detail.

COMPONENTS OF LITTER SIZE

1. The Number of Eggs Shed.

(a) The mechanism of ovulation.

It is well-established that the number of primordial ova in infantile ovaries is greatly in excess of the number normally fertilised during the individual's lifetime. Desaive (1941), working with the rabbit concludes that true ovogenesis probably does not occur at the adult stage. The ripening and shedding of ova are known to be controlled by the gonadotrophic hormones of the anterior pituitary gland. Both the follicle-stimulating and lutealising fractions have been used experimentally to increase fertility, as shown by the wealth of literature on the subject. The anterior pituitary gland is in turn controlled by the diurnal rhythm of light and darkness; recent mouse work on this subject has been reported by Braden and Austin (1954), and Braden (1956).

MacArthur (1942, 1944), selecting for large and small body size in mice, found that litter-size was changed accordingly, due to the greater number of ova shed in the larger mice. While mice of the large line exceeded those in the small line by 24% in body weight, litter-size showed a divergence of 84% in the same direction. This led him to suggest that the two characters may be controlled by common genes, and postulated that the anterior pituitary gland was the link between them.

It has been noted that there is an inverse relationship between the number of ova released from the two ovaries (Runner, 1951; Bloch, 1952; Eckstein and McKeown, working with guinea-pigs, 1955). Hollander and Strong (1950) concluded that unilateral ovariectomy leads to what they called "compensatory hyperovulation" in the other ovary. During the course of some subsidiary work to be described later, we had occasion to count corpora lutea in autopsied pregnant mice. The numbers in the two ovaries were recorded separately, and a negative correlation of -0.41 was observed ($P < .001$).

It is interesting to speculate as to the cause of this inverse relationship. Suppose that the number of eggs to be shed at a particular time is totally determined by, shall we say, some hormonal level in the blood, and that furthermore the eggs are drawn at random from the two ovaries. In such a case, we would expect the number of eggs to be distributed binomially between the two sides, the expectation being equal for each. The "simplified maximum likelihood" method of Robertson (1951) enables us to test the goodness of fit of the data with a binomial distribution. When the test was made, the departure of the data from the expectation based on a binomial distribution was not found to be in the least significant. This finding is compatible with the simple hypothesis that eggs are shed from the two ovaries in a random manner, i.e. were they to be shed sequentially, the "choice" of ovary from which any egg is shed is unaffected by the number it has shed, or not shed, previously.

(b) Age changes

Judging from several references quoted by Hammond (1941), the first litter is submaximal in all the species examined owing to fewer ova being shed. This is not due to the age of the ovarian tissue itself for, as Hammond observes, the transplantation of juvenile ovaries into adult animals causes them to function as adult ovaries. Likewise, immature females when injected with gonadotrophic hormones respond by shedding eggs in large numbers. Hammond concludes that the blood of immature animals lacks sufficient gonadotrophic substances to cause ovulation.

It is the experience of every mouse worker that first litters are smaller than subsequent ones. Yet, two independent pieces of information from this laboratory place this observation in a new light. Falconer (unpublished) found the regression of the size of the first litter on the age of the dam in days to be -0.0016 ± 0.0014. This is obviously totally insignificant. Litter size of course need not be a true reflection of ovulation rate. But Braden (private communication) found that two groups of virgin females, aged 6 to 10 weeks and 10 to 18 weeks respectively, showed no difference whatever in the mean number of eggs shed. This suggests that parity itself has an effect but age is of little consequence, as indeed was observed by MacDowell and Lord as far back as 1925. An interesting physiological problem seems to be involved.

(c) Other factors affecting ovulation rate

In domestic animals, abnormal conditions of the reproductive system, such as cystic ovaries, commonly cause sterility. Similar abnormalities in rodents have been reported by Boyson (1947) and Desaive (1951). It is unlikely that these pathological conditions have a significant effect on the ovulation rate in mice.

Polyovuly is a well-known occurrence in rodents e.g. Davis and Hall (1950), Desaive (1949), but its effect on ovulation rate is almost certainly negligible except perhaps in special cases. For instance, Fekete (1950) found that polyovular follicles were common in the C58 strain of mice. She suggests that a hormonal effect may influence their incidence and that a hereditary factor is likely.

2. The Number of Eggs Fertilised.

As only one sperm is required to fertilise one ovum, and as there are millions of sperm in one ejaculation, it is understandable that some early papers on fertility contain no reference to the contribution of the male. A male was either fertile or sterile. However, with the advance of artificial insemination and experiments with semen dilution, the importance of a sufficiently large number of healthy sperm became increasingly appreciated, and it now appears that there is a margin of some width between complete male sterility and normal fertility.

(a) Sperm production

It has long been known that sperm production ceases with the removal of the anterior pituitary gland, which is therefore of consequence in male fertility also. Once puberty is reached, sperm production does not seem to vary with age for a long time, though some suggest that this question should be re-examined. Kobozieff and Larvor (1953) found that in mice, the number of spermatozoa in the semen decreases progressively after 17 to 18 months of age, but under normal experimental conditions males are usually discarded well before they reach this age.

Sperm may be either deficient in number or abnormal in form, and as indicated above, sterility or lowered fertility may result. Various environmental factors may bring this about e.g. dietary deficiencies, especially vitamin E and also excessively rich protein diets. The effect of temperature is also wellknown, as instanced by rams with woolly scrota that become sterile in hot weather. Hammond (1941) quotes cases where male sterility is caused by infections in, or the blocking of, the tubules of the epididymis.

Glucksohn-Schoenheimer et al. (1949) indicate that some male sterility may be of genetic origin. When normal females were mated to males heterozygous for the t^3 mutation, very few offspring resulted, despite normal copulation. There was some variation between males which the authors attribute to different genetic backgrounds, or to slight differences in the character of the t^3 mutation. Rajasekarasetty

(1951, 1954) examined the semen of heterozygous t^3 males and reported abnormalities affecting the acrosome, shape and size of the nucleus, and the axis of the sperm head. The author also reminds us that morphologically normal spermatozoa may be physiologically inefficient. He believes that, in heterozygous t^3 males, the number of normal spermatozoa falls below a threshold value.

Maqsood (1950) refers to the semen of inbred rabbits which had a poor breeding record. He found that a small percentage of spermatozoa had protoplasmic masses instead of tails. Spermatogenesis was also abnormal in the seminiferous tubules of the testes. A general improvement was effected by thyroid therapy.

These abnormalities have been dealt with at some length, for in many of the sterile matings encountered during the inbreeding stage of the experiment to be described, the fault could be attributed with certainty to the male. Braden (private communication) concurs with the view that this is frequently the case; some inbred mouse semen that he examined was also found to be defective.

The role of enzymes such as hyaluronidase present in the semen is under constant review. We should bear such enzymes in mind as possible instruments of variation in male fertility.

(b) Sperm motility

Good sperm motility has been regarded as one of the most essential characteristics of semen. A recent refinement of the technique of artificial insemination illustrates the importance of motility, namely, the "deep-freeze" conservation of semen, thereby reducing its motility in vitro with consequent improvement in keeping-quality for insemination. But though the refinement is relatively new, its principle has been recognised for many years.

It has often been pointed out that of the many millions of spermatozoa deposited at the cervix during copulation, there is a progressive reduction in numbers until eventually only relatively few reach the site of fertilisation at the upper part of the fallopian tubes. Recent work in this field has been published by Braden (1953), and

Braden and Austin (1954). It therefore appears reasonable that good motility of the sperm is of some importance if coitus is to be followed by successful fertilisation. However, this question is debatable. It seems that spermatozoa do not necessarily ascend the female tract by means of their own motility, but that also the tract itself may actively propel the sperms towards the site of fertilisation. Braden (1953) reviews the subject and concludes that, in the rabbit, both mechanisms may operate. In the mouse, the female tract may have a relatively greater effect, as the sperms are ejaculated virtually direct into the uterus, which is at the time distended with fluid. Two or three contractions would probably disperse the sperms throughout the length of the uterus, although the motility of the sperm itself may become more important once it reaches the tube.

That sperm motility is of restricted importance is shown by El-Sheikh and Casida (1954). They subjected rabbit semen to a number of treatments known to affect motility and inseminated it into does. Their results indicate that fertility is not necessarily dependent on motility, as conditioned by the environment.

There is but scanty information on factors affecting sperm motility in vivo. Bishop and Mathews (1952) found that intravas pH was relatively insignificant, and suggested that the very low intravas oxygen tension, and the deficiency of a carbohydrate substrate, were involved to a much greater degree.

(c) Conditions of the female tract

The third group of factors affecting the number of eggs fertilised is the conditions of the female tract, the importance of which in relation to sperm motility has already been mentioned.

Any abnormal condition of the tract would obviously prejudice fertilisation. Hammond (1941) mentions the presence of inflammations and leucocytes, and the incomplete liquefaction of the mucus of the cervix at oestrus. Krehbiel (1948), working on cervical bypassing and cervicectomy in the rat, concluded that the cervix contributes to the successful maintenance of pregnancy, but that it is not essential for its initiation, development or completion.

In addition to receptive conditions within the female tract, correct time relations between ovulation and mating are of importance if pregnancy is to ensue. Mice probably regulate this themselves; in any case, Genin (1951) found considerable variation in mice in the time taken by spermatozoa to penetrate the ova.

3. The Number of Zygotes Carried to Term.

In general, the number of young born does not exceed the number of eggs fertilised. The exception would be cases of monozygous twinning. The occurrence of such twins in rodents has not been established, but the possibility has been suggested by Gluecksohn-Schoenheimer (mice, 1946) and Cock (rabbit, 1950).

(a) Implantation

Losses of blastocysts before implantation are common; reasons for these do not seem to have been investigated. Mouse blastocysts normally implant on the fifth or sixth day. For this to happen, the uterine mucosa has to be in a receptive state, a condition brought about by the progesterone secreted by the corpora lutea and placentae. If progesterone is deficient, implantation is delayed; this happens regularly in lactating females. Hollander and Strong (1950) conclude that there is no spacing agency for implantation sites other than the churning of the uterus and chance distributions

(b) Maintenance of pregnancy

Progesterone is necessary also to maintain pregnancy for a time after implantation, the exact period depending on the species. In the mouse and rat, the corpora lutea persist right up to parturition, and progesterone is required throughout. The amount of luteal tissue required for the maintenance of pregnancy in the rat has been reported on by Kelsey and Meyer (1950). They found that pregnancy was maintained when all but two corpora lutea were removed on the eighth day of pregnancy. This seemed to be their lower limit, for when all but one corpus luteum were removed, even on the fifteenth day, pregnancy was only partially maintained.

The importance of the placentae as secretors of progesterone in the rat is

illustrated by Selye et al. (1935) and Haterius (1936). They found that pregnancy is maintained following the removal of the ovaries shortly after mid-pregnancy if all but one of the foetuses are removed, provided that the placentae of the removed foetuses are allowed to remain in situ.

(c) Foetal mortality

Embryos may die from either genetic or non-genetic causes. Examples of the latter are certain infectious conditions that have to be rigorously controlled in domestic animals if fertility is to be maintained at a reasonable level; but their effect on mouse fertility is probably small, if even they exist.

Foetal mortality in rodents shows a pronounced maternal effect in that the mortality rate rises with age of mother. Hollander and Strong (1950), in their comprehensive investigation of intra-uterine mortality in the mouse, found that females of more than twelve months of age showed a significant decrease of about 15% in the number of live embryos, and that this is due to increased foetal mortality rather than the number of eggs shed. Wanke (1938), who worked on the impressively large scale of 7916 litters of mice, struck the same phenomenon. His largest litter was the second; a progressive decline in litter size followed until the sixth litter actually became smaller than the first owing to increased mortality of the young. Murray (1934) reports a similar result. Frazer (1951, 1955) examined the position in the rat, with similar conclusions. He notes that the losses were associated with heavier mothers, stating in parenthesis that they are therefore the older ones.

Hollander and Strong (1950) found the average mortality rate to be 15%, and that this was remarkably constant in their heterogeneous material; it rose to 25% in females more than one year old. The authors conclude that mortality occurs at every stage of gestation, but that 72% of it probably occurs two to three days after implantation. Mortality was not significantly related to the degree of crowding in the uterine horn. Frazer (1951, 1955), working with the rat, came to the opposite conclusion regarding the effect of crowding. He found that the loss both of whole

litters and of individuals before the ninth day was associated with unusual numbers of ova, both high and low. Small litters on the ninth day usually died later.

Lethal genetic factors that kill embryos are universally distributed, and many are known for the mouse alone. For instance, Kirkham knew about the homozygous yellow lethal as far back as 1919. Though the list of these lethals and semi-lethals is now formidable, we should not, nevertheless, overestimate their importance. Hollander and Strong (1950), while agreeing that some mortality has a genetic basis, concluded that recessive lethals are of minor importance, as F_1 embryos from strain crosses showed a barely significant reduction in mortality from the general rate.

Schilling (1952), who studied the cause of pre-natal mortality in the rabbit, suggested that hereditary lethal factors became operative only when organ differentiation commences; he blamed untimely contraction of the uterus and hormonal unbalance for many of his deaths.

NON-GENETIC VARIATION IN LITTER SIZE

The above analysis of the major factors controlling litter size in the mouse shows that it is a complex character. It is reasonable to suppose that it is influenced by many environmental factors. Falconer (1956) has found that the heritability of the character is rather low. In view of this, we must examine briefly some of the non-genetic variation to be found in litter size.

(a) Seasonal effects

In view of the effect of daylight on the activity of the anterior pituitary gland, a seasonal fluctuation in litter size could reasonably be expected. Seasonal effects on litter size are not easily measured in wild populations, and the breeding season is normally limited and well defined. Davies and Hall (1950), working on wild rats in Maryland, U.S.A., found no seasonal effects on reproduction in large females, but that pregnancy was more frequent in small females in the spring and autumn; litter size was unaffected throughout. Bluhm (1947) found a similar effect in laboratory albino

mice; while conception rate was highest from June to August and lowest from October to January, litter size did not vary with the season.

Parkes (1924) found rather a different effect; he attained his largest litters at the end of the summer. Parkes mentions also that where laboratory mice are kept in quarters where the temperature is not controlled, the breeding season is limited to the summer months, as in wild mice. He mentioned that this cessation of breeding during the winter months was probably a temperature effect; in this he is supported by Bluhm (1947).

The only pertinent data on seasonal effects as they affect our experiment are those collected on the same stock in this laboratory, summarised in Figure 3. This shows a plot of the generation means of the unselected control stock against the month of the year during which the particular generation was born. The data extend over a period of 3½ years. Rather surprisingly, there is no apparent seasonal or other trend. A test of the justification of this conclusion comes from the analysis of variance of generation means.

	d.f.	Sum of squares	Mean Square
Between generations	15	78.677	5.245
Within generations	474	1956.933	4.129
Total	489	2035.610	

$$F^{15}_{474} = 1.27 \qquad P > .20$$

This shows that the variation from generation to generation is no more than could be accounted for by sampling error arising from the variation within generations. This takes account not only of seasonal changes but also variation due to any other environmental cause.

The season of the year may of course still affect conception rate. But there is no evidence from the graph that the generation interval is longer during any particular season. In the absence of such an indication, a more critical examination of the data

was omitted.

It follows that if there is any seasonal variation in the reproduction of mice under controlled laboratory conditions, then it must be very small.

(b) Parity

The fact that the first litter is sub-maximal has been noted earlier. After the second litter however, there is no further increase. Parkes (1924), Murray (1934), Wanke (1938) and Russel (1954) all found that after the second or third litter, litter size gradually diminishes. This is due, at least in part, to the increased incidence of foetal mortality.

It is possible that second litters are biased upwards through selection against low yielders in the first litter. This apparently is not so, as shown by data from the EV and Z stocks referred to previously. EVE and EVC do not differ in this respect, and the data from them is pooled. When first litters with paired second litters are compared with all first litters, no departure is revealed.

	EV	Z
All first litters	8.53 ± 0.11	7.01 ± 0.12
First litters with paired second litters	8.53 ± 0.11	6.90 ± 0.12

(c) The effect on pregnancy of concurrent suckling

Mice commonly suckle one litter while they are pregnant for the next, but apart from the delay in implantation, the unborn litter does not appear to be affected in any way by the extra demands made upon its dam. Certainly no effect on the size of the litter when born is established, a conclusion supported by Bruce and East (1956). The second litters from the EV data could be divided into two classes, as follows:-

	No.	Litter size	Variance of litter size
Pregnancy with concurrent suckling	147	9.547	9.297
Pregnancy without concurrent suckling	44	9.844	6.180
Probability that the difference is due to chance		$\sim .50$	$\gtrsim .05$

Though litters where suckling is concurrent are both smaller and more variable, neither difference is significant. If there is no reason to expect these litters to be more variable, then both tails of the distribution of z must be considered, and the probability that the difference in variance is due to chance is thereby doubled.

(d) The greater variance of second litters

It is common experience that second litters have a greater variance than first litters. This was also found to be the case in the EV and Z data. However, this is due entirely to the higher mean of the second litters, as shown by the good agreement of the coefficients of variation in both stocks

Stock	Variance of 1st. litter	Variance of 2nd. litter	C.V. of 1st. litter	C.V. of 2nd. litter
EV	7.13	8.55	0.31	0.30
Z	6.54	9.34	0.36	0.36

(e) The effect of age of dam on litter size

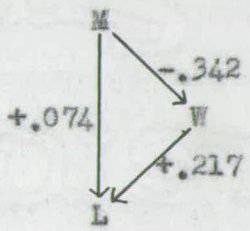
Reference has been made earlier to some unpublished data of Falconer on litter size and Braden on egg counts, showing that for first litters, the age of the mother does not affect litter size. This has also been reported by MacDowell et al. (1929), and by Eckstein and McKeown (1955), the latter on guinea-pigs. For experimental purposes, this is very expedient. It means that all the matings of a generation can be set up on the same day without causing any additional variance to litter size, even though there is a little variability in the age of the mice at mating. At the same time, the implication of the next paragraph is that this will not necessarily hold if the mice are actively growing at the time.

(f) The effect of body weight of dam on litter size

Within any stock of polytocous animals, it is usual for the larger females to bear, on the average, larger litters. Some experimental evidence on this is quoted by MacDowell et al. (mice, 1929) and Eckstein and McKeown (guinea-pigs, 1955). The latter observed a positive correlation of 0.22 between litter size and maternal weight,

which was virtually unchanged when maternal age was held constant. Though their material is too heterogeneous to place great reliance on actual figures, they concluded that this correlation is determined mainly by the positive correlation between weight and the number of ova produced.

But in a breeding experiment, the direct effect of body weight of dam on litter size does not end here. A large mother gives a large litter which is consequently small at weaning - a handicap which is still reflected in its weight at mating time. The daughter of a large mother is therefore small, and produces a small litter when it in turn bears offspring. The nett average effect is thus a negative regression of litter size on the size of the litter in which the dam was born, unless there also exists the positive genetic pathway expected in a heritable character. These complicated interactions have been unravelled by Falconer (1956) by partially regressing litter size (L) on the body weight of the dam (W) and the size of the litter in which the dam was born (M).



The figures are partial regression coefficients.

This diagram gives the "cause and effect of the interrelationships. The mother's body weight is inversely correlated with the size of the litter in which she was born, and directly with the size of its own litter. The product of these two coefficients is -.074, which would give the regression coefficient of litter size on maternal litter size, if no other pathway were operative. There is however a direct genetic pathway which is measured as the partial regression of litter size on maternal litter size holding the mother's weight constant. This coefficient is +0.074, as shown. The regression of daughter on dam represents, of course, half the heritability, and the figure obtained agrees remarkably well with what is observed by selecting within litters,

thereby holding mother's weight constant (Falconer, 1956).

We see therefore that litter size is affected by maternal litter size through two independent determinants of equal magnitude but opposite sign. This explains why the direct regression, when measured, comes out to be zero (Falconer, 1956).

PREVIOUS WORK ON INBREEDING AND CROSSING

It was suggested earlier that no experiment has been done that is strictly comparable to ours. Nevertheless, there are several references to rodent work that indicate what we might expect. The general conclusion is that the fertility of rodents shows a marked inbreeding depression, with the complementary increase on crossing. Crossing without inbreeding has also been known to increase fertility, probably reflecting a certain amount of homozygosity in the material used.

From the examination of the components of fertility, it is readily seen how inbreeding might affect litter size at several different points. It is not surprising therefore to find that inbreeding of the dam and of the litter itself are both reflected in litter size, though apparently with different force in different circumstances. An attempt was made to evaluate the relative importance of these effects in our case.

The earliest well-analysed rodent work on this subject is that of Wright (1922a,b) on guinea pigs. Wright found that fertility declined with inbreeding, particularly in the early stages. When he crossed his surviving lines, the first crosses showed little increase, but crossbred mothers had a much higher litter size. This led Wright to conclude that litter size is almost wholly a maternal character.

Wright is supported in this contention by Green (1931), working on the mouse, though Green's experiment was rather different. He crossed two mouse species, Mus musculus and Mus bactrianus, that differed characteristically in litter size. The results of the species crosses and back-crosses indicated to Green that litter size is largely determined by the mother, and is significantly augmented by crossbreeding in the mother.

The fertility of crossbred mice has often attracted attention. Castle (1926) was aware of the phenomenon. More recently, Grüneberg (1939) found fertility in some crossbred mice the like of which he had not encountered elsewhere. But in mice at least, the genetic constitution of the litter also is often of some consequence.

Fortuyn (1932) crossed two strains of albino mice differing in fertility, and found that both strains reared a larger number of offspring when the litter was hybrid than for pure strain litters. However, he did not measure the size of the litter until the mice were one month old, and Wright (1922) had noted that the percentage reared of young born alive showed an immediate improvement on crossing, namely in F_1 litters with inbred mothers. Fortuyn's results do not therefore throw much light on the effect of the litter's genotype on litter size at birth.

More indisputable is the evidence of Forsthoefel (1954). He split litters of the BALB/C inbred strain; some females were mated to their brothers while their litter mates were outcrossed to inbred males of another strain. The former had a litter size of 4.82, the latter 6.82, the difference being very significant. He suggests that this is brought about by the masking of recessives that reduce uterine viability. He comments on the discrepancy between his results and those of Wright and Green, and argues that it may be due to the greater variability of the material and environment of the earlier workers. This would cause any small effects of the genotype of the young to be obscured by the interplay of other factors. His results are so marked because his experiment was designed to test this one variable.

Possibly the most comprehensive study of the effects of inbreeding and crossing on litter size in mice is that of Eaton (1941, 1953). His material again differed radically from ours. He gathered several inbred strains and made crosses to test the effect on litter size. About half the crosses (F_1 litters) showed fertility superior to that of both parent inbred strains, though some crosses were even inferior. The combination of three inbred strains, using a hybrid dam, gave a greater increase in

fertility. Eaton also produced some inbred lines from crossbred material and crossed these. He found that the effect of the heterozygosity of the litter was much reduced if crosses were made following less than six generations of inbreeding.

It seems therefore that the genotype both of dam and of litter affect litter size, but that in general the former is of greater relative importance. It does not appear that the effect of inbreeding on male fertility has been examined.

There is also the unpublished work of Falconer on the effects of inbreeding on fertility. This is of importance, as the work was done in the same laboratory and on the same stock as was used for the experiment to be described. The results are summarised in Figure 4. Twenty inbred lines were started, the first generation being a double-first-cousin mating; thereafter inbreeding was continued by brother-sister matings. Ten lines were propagated without any selection whatever, and are designated JU; in the other ten, JS, inbreeding was accompanied by selection against small litters. It is interesting to note that selection was completely ineffective in arresting the inbreeding depression. After about three or four generations of inbreeding, fertility was so much reduced that several lines were completely lost.

Figure 5 shows what happened to the variances of the inbred lines. Contrary to the theoretical expectation, the component of variance between lines appeared to decrease, while the within line variance if anything increased. The fluctuations from generation to generation are perhaps too great to place much confidence in these trends. Nevertheless, this increased variance within inbred material seems to be a common experience. Wright (1949) wrote, ". . . genetic uniformity is compatible with great phenotypic variability, since accidents of development and environmental influences are, of course, not controlled in this (genetic) way." Instances of the phenomenon in the mouse have been reported recently by Grüneberg (1954), McLaren and Michie (1954) and Hamer (1955).

This then was the information we possessed on the effects of inbreeding and crossing on litter size in mice at the time our experiment was launched. How it affected the design of the experiment is described in the next chapter.

III. EXPERIMENTAL MATERIAL AND METHODS

It has been stated earlier that the major point of investigation was to be the influence on litter size of crossing unselected inbred lines. This means that ideally, neither artificial nor natural selection against litter size should be operative during the inbreeding stage of the experiment; in other words, none of the lines set up from the original outbred population should be lost. Unfortunately, natural selection can not be excluded. Reference has been made to the experience of Dr. Falconer with the JU and JS inbred material. This indicated that it would be impossible to carry the inbreeding coefficient beyond about .50 without introducing the probability of losing lines through low fertility or even complete sterility. It was clear therefore that the crossing would have to be done using partly inbred material.

Though this was forced upon us as an experimental expediency, its obvious theoretical disadvantage was to some extent mitigated by greater practical application. For the difficulty and cost of breeding and maintaining inbred lines becomes prohibitive in farm animals, even in pigs (see, for instance, Donald, 1955). In view of this, the possible use of partly inbred material must be explored.

The broad outline of the experiment was therefore as follows. The inbreeding stage was confined to three generations of brother-sister mating. The lines were then crossed at random, giving crossbred litters. As litter size is so largely a maternal character, these crossbreds had to be mated to test their fertility, for this was what the experiment was calculated to determine.

INBREEDING PROGRAMME

It was decided to start with thirty inbred lines. A good number of lines was required to reduce sampling errors in reconstituting the crossbred population later. The original material consisted of surplus mice from the selection experiment for litter size, described by Falconer (1956), where he also describes the previous history

of the stock, designated "J". The selection experiment contains three strains - large litter size (JH), small litter size (JL), and an unselected control stock (JC), which was also used as the control for our experiment. By the time mice were required for this work, selection in the original stocks had proceeded for ten generations. It would perhaps have been advantageous to start all the inbred lines from the unselected stock, JC, but this would not have provided nearly sufficient unrelated lines. We were therefore obliged to take mice from the three stocks, JH, JL and JC. Ten litters were taken from each stock; each litter came from one family and was to be the foundation of one inbred line. None of the lines were closely related. The highest and lowest litters from JH and JL respectively were required for the selection experiment. When choosing litters for our work, this bias was counteracted by rejecting also the other extreme. With this exception, and the avoidance of sib litters, the foundation litters were chosen at random.

The inbred lines were propagated in the following manner. All the available females of a litter were divided between two of their sib males, a precaution against male sterility or accidental loss. Each line thus normally gave birth to more than one litter. One of these litters was chosen at random and the process repeated. The random choice was occasionally disturbed by a litter not containing the required two males and two females; it was excluded from the choice where possible, in the interest of safeguarding the line. It might be that this introduced a little selection against litters of extreme sex ratio and against some small litters. However, Falconer (1954) has showed that the first of these is ineffective, and we have seen from the JS and JU data (see chapter 2) that the same is also true of the second. It is considered therefore that this probable selection is of no consequence.

For recording purposes, the lines were numbered serially from 1 to 30. Lines 1 to 10 came from the JH stock, 11 to 20 from JL and 21 to 30 from JC. Mice were mated when the youngest were six weeks old. The oldest were usually about 8 weeks by this time. Generations were kept in step, within the three major groups. When they

were first obtained, JH and JL were contemporaneous, but JC lagged three to four weeks behind. In order to standardise roughly the age at mating, they were kept this way. One attendant advantage of this was the consequent staggering of recording work.

In spite of every reasonable effort to maintain them, four lines failed to complete the inbreeding stage and, of course, are not represented in the crosses. This however is not quite as bad as it may seem, for one of the lines lost, line 29, was a good one and became extinct through misadventures in no way connected with its fertility. Another, line 25, also produced good litters, but at one stage they lagged three weeks behind their youngest contemporaries; it was decided that the time involved was more valuable than the line, which was therefore dropped. But the loss of the other two lines, 9 and 20, can be ascribed, certainly in part, to low fertility. Each gave birth to small litters, all of which died before weaning.

There was therefore undoubtedly a little selection of inbreds after all. In addition, it must be pointed out that line 14 also is but weakly represented in the crosses, as will be seen later. Nevertheless, it seems reasonable to suppose that the effect of this selection is not sufficiently large to alter materially the main conclusions that emerged from the work.

CROSSING PROGRAMME

Ideally, each line should be crossed to all the other lines to form an orthogonal set of diallel crosses. In view of the numbers of mice and cage space that this would require, it was obviously out of the question in the present experiment. Another system of crossing was therefore necessary.

As the three major groups were of slightly different origin, it was decided to do all the crossing within such groups, i.e. lines 1 to 10 would be crossed inter se and to none of the others; likewise, lines 11 to 20 and 21 to 30. Crossing between the different groups would introduce an additional variate from which, as far as this experiment was concerned, little useful information would derive.

It became clear that whatever system of crossing was adopted, an answer would be obtained to the main question posed earlier, namely, how does the crossbred population compare with an outbred one. The requirements of the crossing programme were therefore to provide as many supplementary data as possible, within the limits imposed by exigencies of cage space. It was decided to attempt to form an estimate of the general combining ability of the lines, specific combining ability, and maternal effects. The first two would be reflected as variance components, the third as the difference between reciprocal crosses.

In order to use all available lines as both male and female parents, pair matings were employed. The size of the litter of any one pair was the estimate of the value of that cross. A certain number of duplicate crosses was therefore required to estimate the error variance i.e. the variance between replicates of the same cross.

It was considered that in order to obtain a reasonable estimate of a line's crossing ability, it should be crossed to four others, strictly at random. The principle of the scheme of crossing eventually chosen is illustrated in Figure 6. The number in each cell is the number of matings of that type that would have been set up had the mice been available. If the mice were not there, no other type of mating was substituted, as this would affect least the randomisation of the crossing; in any case, some wastage was expected if the scheme was to be housed in the available cage space. It was calculated that mice could be supplied for only about half of the theoretical number of duplicates, but this would still leave about 30 degrees of freedom, over the three groups, for estimating error.

The actual numbers of matings which were set up in the three groups is shown in Figure 7. As will be seen from the next section, only a few of these failed to produce a litter.

This then describes the first cross. But a point of greater interest is the fertility of the crossbreds. A crossbred female was therefore mated to a crossbred male in a random manner, except that mating between mice with a common parental line

was avoided. Thus, a female from a cross between, say, line 1 and line 8 was not mated to any male derived from a cross involving either of these two lines. Every F_2 litter was therefore a "four line" cross. As more cage space was available than for the first cross, a certain number of triplicates was set up, as shown in Figure 9. This was done as the error variance in the first cross was rather large.

The analysis of the second cross data was carried out on the same scheme as the first cross. In the analysis of the second cross, litter size is treated as a maternal character, in that the direct effect of the male on litter size in fertile outbreds was known to be negligible (Falconer, 1956). In other words, the male was purely the means whereby the litter size of the female could be measured. We are interested in the effect on litter size of the genotype of the mother, as determined by her inbred parents.

The inbreeding of parents and offspring are of course "out of step" throughout the course of the experiment. To avoid confusion later, the following table shows the inbreeding coefficients of parents and offspring for every generation. The foundation mice from the outbred stocks are designated JO, the inbred lines - JR, and the crosses - JRX.

Generation	F_p	F_o
JO	0	0
JR_o	0	.25
JR_1	.25	.375
JR_2	.375	.50
JR_3	.50	0
JRX	0	0

where F_p is the inbreeding coefficient of the parents
and F_o " " " " " " offspring

Throughout the course of the experiment, the following measurements were taken.

(i) number of live young

(ii) number of dead young found

(iii) number of young weaned

In these three recordings, the sex ratio was also noted

(iv) six-week weight of dams

(v) post-partum weight of dams.

Weights were recorded chiefly because of the known effect of mother's body weight on litter size.

SUBSIDIARY EXPERIMENT

In an earlier section, litter size was regarded as a composite of three major components, - the number of eggs shed, the number of eggs fertilised, and the number of zygotes carried to term. It was decided to attempt to see how these three components react individually to the effects of inbreeding and crossing.

What measurements were to be used? Unless very large numbers are to be employed, the measurements must be taken on the same animal, which can only be done by killing the female in late pregnancy.

At this time the corpora lutea are large and with practice, they can be counted with moderate ease. This will give a good estimate of the ovulation rate, though factors such as polyovuly may have a slight influence. The migration of blastocysts from one horn of the uterus to the other is also known to occur (McLaren and Michie, 1954). This will not affect the animal as a whole, but it will give a discrepancy between the two sides of an animal, which may or may not be detectable.

In late pregnancy, the number of live foetuses must correspond closely to the number carried to term. Any mortality that occurred after implantation will also be detectable as resorptions, the smallest of which are sometimes referred to as moles. Hollander and Strong (1950) were of the opinion that these moles are not lost through complete resorption, but there is no critical evidence on this point. It is therefore

possible in theory that evidence of some very early post-implantational mortality might disappear without trace.

It was thus possible to measure reasonably well the first and third of the three components. The second, the number of eggs fertilised, is more elusive, and only the examination of the eggs themselves could provide the complete answer. All that could be recorded by the method employed in this work was the difference between the ovulation rate and implantation rate. It seems likely that the bulk of this loss would be due to failure of fertilisation, though the frequency of pre-implantational mortality of blastocysts appears to be uninvestigated. In any case, by taking the three measurements, - ovulation rate, implantation rate and live embryos - we can at least see at what stage of reproduction inbreeding depression is operative.

The experimental method adopted was to take five groups of mice, with about 25 to 30 matings in each. The first consisted of inbred parents with inbred young, the second inbred parents with crossbred young. In the third group, inbred females were mated with unrelated crossbred males; group IV was its reciprocal. For the fifth and last group, the parents were unrelated crossbreds. The following table gives the inbreeding coefficient of parents and offspring in each group.

Group	♀ parent	x	♂ parent	F_{pf}	F_{pm}	F_o
I	Inbred A		Inbred A	.50	.50	.59
II	Inbred A		Inbred B	.50	.50	0
III	Inbred		Crossbred	.59	0	0
IV	Crossbred		Inbred	0	.59	0
V	Crossbred		Crossbred	0	0	0

where F_{pf} is the inbreeding coefficient of the ♀ parent
F_{pm} " " " " " " ♂ "
and F_o " " " " " " offspring

All the material came from surplus mice not required for the main crossing programme. It is therefore selected in favour of fertile lines. If this has any effect, which is very doubtful, the data should still be comparative, as there is no

reason to suppose that the bias does not affect all groups equally.

The females were checked daily for vaginal plugs, and killed when 16 days pregnant. This time was chosen as it seemed to be sufficiently near to the end of pregnancy, while the corpora lutea counts would not be affected by maturing follicles, which would make the task more difficult. The following data were collected, right and left sides being recorded separately.

(a) number of corpora lutea

(b) number of implantation sites

(i) Moles - early postimplantation mortality

(ii) Resorbing embryos - later mortality

(iii) Live embryos.

(i) and (ii) both showed low rates of mortality and were pooled for analysis.

Groups I and II were synchronous. So were Groups III, IV and V, but one generation later than the first two groups. In addition, each group was somewhat staggered, about 20 matings preceding the remainder by four weeks or so. There is therefore a certain amount of temporal variation within groups, with perhaps rather more between Groups I and II on the one hand, and Groups III, IV and V on the other.

IVA. RESULTS

THE EFFECTS OF INBREEDING AND CROSSING ON LITTER SIZE

The data that accrued from the experimental work will be presented in four sections in the following order:-

A. The effects of inbreeding and crossing on the mean litter size.

B. The differentiation between inbred lines in litter size.

C. The analysis of variance of litter size in crosses between inbred lines.

D. Subsidiary observations

(i) Six-week weight of females

(ii) Mortality observed at birth

(iii) Weaning rate.

The results of the subsidiary experiment are given separately.

A. MEAN LITTER SIZE

It has been shown earlier in a review section how the inbreeding both of dam and of the litter itself depress litter size. It would therefore be desirable to consider these two effects separately, but this is possible only to a limited extent. In the first generation of full sib mating, JR_0, any reduction in litter size is clearly attributable to inbreeding in the young, as the parents are still outbred. Likewise, in the first cross, JR_3, any increase will be due to crossbreeding in the young. In the second cross, JRX, a further increase in litter size would be attributable to crossbreeding in the parents, as the heterozygosity of the young would not be expected to differ from the previous generation. For the remainder of the experiment, inbreeding of dam and of offspring proceed simultaneously but at different stages, the inbreeding of the dam lagging one generation behind that of her offspring. The dual determination of the character will therefore render the interpretation of the data somewhat less precise, and at times, only an empirical treatment of the results will be possible.

Maternal effects on litter size add further complications. If inbreeding reduces litter size, then these small litters have an advantage in milk supply which will be reflected in their six week weight. We have seen in an earlier section how this will affect their fertility and result in larger litters in the following generation. In other words, the deleterious effects of inbreeding are being counteracted. The greater the inbreeding depression in litter size, the greater the compensation in growth rate, and thereby in litter size in the following generation. Though inbreeding may depress litter size, an attendant maternal effect simultaneously increases it. However, Taylor (unpublished) has shown by working with a standardised litter size, how six week weight itself declines on inbreeding, partly no doubt due to a decline in lactation or maternal performance. The decline in weight causes a maternal effect which leads to smaller litters, thus counteracting the maternal effect noted above.

We therefore have two maternal effects acting in opposition, and the observed result will involve a balance between the two.

Theoretically, the complications due to maternal effects could have been overcome by standardising litter size at birth in the experiment described here. But with inbred mice, whose expected fertility is low, this is not an attractive practical proposition. The "standard" would need to have been a low one, and this would have had serious repercussions on the survival of the lines. It was shown in the previous chapter that even without imposing the additional strain of standardising litters, the policy of maintaining all inbred lines was only partially successful.

We shall now consider the generation means for litter size during the inbreeding and crossing phases of the experiment. They are shown below in tabular form, and are illustrated graphically in Figure 9.

TABLE I

LITTER SIZE - GENERATION MEANS

Generation	JO	JR_o	JR_1	JR_2	JR_3	JRX
Inbreeding coeff. of parents	0	0	.25	.375	.50	0
Inbreeding coeff. of offspring	0	.25	.375	.50	0	0
Group H	9.11	7.11	5.82	5.82	7.03	9.29
L	7.41	6.47	6.11	5.14	5.91	8.65
C	7.84	6.60	5.52	6.12	5.67	7.47
Unweighted mean of 3 groups	8.12	6.73	5.82	5.69	6.20	8.47

Group H refers to lines 1-10; these mice had originally been selected for large litters. Likewise, L refers to lines 11-20 (from the low line of the same experiment), and C to lines 21-30 (from the control stock).

From the four points available for examination, it appears that litter size declines on inbreeding in a linear fashion, in relation to inbreeding coefficient. The only suggestion to the contrary comes from the mean of lines 21 to 30, which increases during the last generation of inbreeding and falls again when the lines are crossed. This does not agree with expectation, nor with the behaviour of the other two groups. The most likely explanation is that the point for the JR_2 generation is spuriously high through sampling errors. It will be recalled that this group lagged three to four weeks behind the other two. It might therefore have been subject to some particular short term environmental influence that did not affect the others.

In the first generation of inbreeding, mean litter size fell by 1.39 as a result of increasing the inbreeding coefficient of the young from zero to 0.25. Over the next two generations, there is a further fall of 1.04 in mean litter size; as indicated earlier, it is not possible to determine to what extent this is due to further inbreeding in the young, and to what extent it is caused by inbreeding in the parents.

The effect of crossbreeding is introduced in two stages, first in the offspring and

then in those offspring used as parents. As litter size is largely governed by the mother, it is not surprising that the bigger increase should accompany the second phase of the crossing. When the offspring are crossbred but the parents inbred, i.e. in the JR_3 generation, litter size is increased on the average by a mere 0.51 of a mouse over the previous generation. But when these crossbreds are mated inter se, a further increase of 2.27 results from the effect of crossbreeding on the dam.

The mean litter size of the crossbred mice used as parents, generation JRX, is 0.35 of a mouse higher than the original outbreds, JO. The comparison of these two means is of prime importance, and represents the major interest of the whole experiment. The difference of 0.35 is not quite significant at the 5% level, despite the slight involuntary selection, mentioned earlier, that was introduced during the inbreeding phase of the experiment. During the period of the experiment, the mean litter size of an unselected control stock varied between 7.00 and 8.17. In comparison with this variation, a difference of 0.35 appears unimportant. There is therefore no reason to suppose that the mean of a crossbred population, obtained by crossing unselected inbred lines, would differ from that of the outbred population from which the inbreds were derived. This of course is what would be expected from theoretical considerations, but the point does not seem to be universally appreciated.

There is one anomaly in the data, concerning the effect of inbreeding and crossing on the litter. In the first generation, JR_0, while the mothers remained outbred, the coefficient of inbreeding in the young increased from 0 to .25; this resulted in an average decrease in litter size of 1.39. In the JR_3 generation, the inbreeding coefficient of the young was decreased from 0.5 to 0. Extrapolating from the results of the first generation, the expected inverse change in litter size would be 2.78; the observed change was 0.51. Here, of course, the mothers were inbred, and the discrepancy suggests some form of interaction between the inbreeding of the dam and of the litter. In simple terms, inbreeding may impose a limit on a dam's potential fertility,

and no amount of heterozygosity in the young would increase the size of litter above a certain level. At the other end of the scale, in outbred dams, any reduced viability in the unborn litter would be fully revealed in the reduced litter size at birth.

As the mean litter size of outbreds and inbreds is different, this discrepancy is magnified somewhat by a scale effect. Furthermore, it must be noted that the increase in litter size when the young are crossbred is 0.51 compared with the previous generation. The parents are now further inbred, and had the offspring been inbred as well, we can estimate that litter size in the JR_3 generation would be somewhere in the region of 5.0, assuming a linear decline. The effect on litter size of the crossbreeding of the young is therefore greater than the increase actually measured, but the decrease when the litter is first inbred is still markedly greater.

The small increase in litter size when the offspring were crossbred compared to the much larger increase when the dams were crossbred was noted also by Eaton (1953), if the inbreeding had proceeded for less than six generation. In the present experiment inbreeding had proceeded for three generations only, and the result agrees with Eaton's observation.

These then are the effects of inbreeding and crossing on mean litter size in mice. The general impression is the expected one of a decline on inbreeding with the corresponding recovery when the lines are crossed. Further, the mean litter size of the crossbred mice is at the same level as the mean litter size of the original outbreds. The pattern of response was similar in the three major groups that constituted the experimental population, despite occasional changes in order of mean performance.

B. DIFFERENTIATION BETWEEN INBRED LINES

The classical theory of inbreeding indicates that inbred lines will become differentiated, with a corresponding increase in uniformity within the lines. The mathematical expressions are $2F\sigma_g^2$ for the between line variance, and $(1-F)\sigma_g^2$ for the within line variance, where σ_g^2 is the additive genetic variance in the initial

population and F is Wright's coefficient of inbreeding. At complete inbreeding, the initial genetic variation is thereby doubled. However, these expressions are true only if the genes act additively; they will not hold for dominance and epistatic deviations, and in most instances, the observed result on inbreeding will differ from these expectations.

The theoretical treatment of the effect of inbreeding on variation due to dominance and epistasis has not been developed fully, but Robertson (1952) has done this with respect to variation due to recessive genes. He showed that the within line variance in such a case would increase on inbreeding until F is in the region of 0.5 and then decline, and that the between line variance will also increase, but only slowly during the initial stages of inbreeding, as the increase is proportional to F^3. Robertson shows also that the same general conclusion will probably apply to genes showing over-dominance.

It appears therefore that in an unknown genetic situation, changes in within and between line variances are unpredictable. For this reason, every empirical observation is of some value. The results obtained from the present experiment are summarised here in tabular form. The figures refer to litter size during the first three generations only. The data from the JR_3 generation are not applicable as the offspring are crossbred, and as will be shown shortly, the variance in litter size can not be partitioned in a simple manner into within line and between line components.

TABLE IIa

LITTER SIZE - WITHIN LINE COMPONENT OF VARIANCE

Group	JR_0	JR_1	JR_2
H	5.67	7.68	2.16
L	6.54	7.37	3.84
C	3.36	5.04	3.48
Unweighted mean	5.19	6.70	3.16

TABLE IIb

LITTER SIZE - BETWEEN LINE COMPONENT OF VARIANCE

Group	JR_0	JR_1	JR_2
H	3.10	1.56	4.48
L	1.34	-1.35	3.17
C	0.02	0.03	0.79
Unweighted mean	1.49	0.08	2.81

These results are illustrated graphically in figure 10.

With three points, it is clearly impossible to establish a trend in any of these figures. The only statement that can be made with confidence is that although there is clearly differentiation between the lines, neither the within nor the between line component of variance in litter size changed materially during the inbreeding phase of the experiment.

C. ANALYSIS OF VARIANCE IN CROSSES

It has been shown earlier how the effect of crossing was introduced in two stages, each measuring a different effect. In the JR_3 generation, the parents were unrelated inbreds, so that the litter was crossbred. Any increase in litter size was therefore due to the increased viability of the unborn young and to no other cause. Every line was used both as a female and as a male parent. The component of variance between lines as female parents ("between dam-lines" for brevity) therefore represents differences between lines in ovulation rate and in the effect of the dam-line on the survival of the young. The latter effect, as far as the component of variance is concerned, may be either of additive genetic or non-genetic origin. The component of variance between lines as male parents ("between sire-lines") represents differences between lines in the fertilising capacity of the sperm and in the sire-line's additive genetic contribution to the viability of the young. The interaction component of variance represents dominance and epistatic "nicking" effects on the viability of the young, and

compatibility relationships, if they exist, between sperm and egg at fertilisation. All this refers to the first crossbred generation, JR_3.

In the JRX generation, these crossbreds are used as parents. As the average heterozygosity of the young in a four-line cross is unchanged from the previous generation, any further increase in litter size is due entirely to the effect of crossbreeding on the parents. Falconer (1956) showed that the effect of the sire on litter size in fertile outbreds is negligible. We are therefore able to treat litter size in this generation as a character wholly determined by the mother. As indicated in the previous chapter, the female's litter size is the measure of the cross between her parents. The analysis of the JRX generation is therefore the same as that of the JR_3 generation, as the structure of the crossing programme is the same in both. This structure may be illustrated diagrammatically as follows:

Generation	Crossing	Measurement
JR_3	Line A x Line B ↓ Cross AB X C x D ↓ CD	Effect of crossbreeding in LITTER
JRX	4-line cross (measurement of cross between A and B)	Effect of crossbreeding in DAM

What do the components of variance measure in the second cross, JRX? The between dam-line component measures the variation in the additive genetic contribution of lines (used as female parents) to the fertility of the crossbreds. The between sire-line component measures precisely the same, the lines in this case being used as male parents. The interaction component again measures dominance and epistatic nicking effects, this time on the fertility of the crossbreds.

A subsidiary objective of the experiment was to attempt to evaluate the variation in the general combining ability of the lines and in the special combining ability of

the crosses. The "general combining ability" of a line refers to the average performance of the crosses between that line and all other lines. The "special combining ability" of a particular cross refers to the difference between the performance of the cross and what would have been expected from the general combining abilities of the parent lines. The variation in the general combining ability of lines will be represented in the present analysis by two components of variance, between dam-lines and between sire-lines. The variation in special combining ability will be the interaction component of variance.

What variation in general and special combining abilities are we likely to encounter? It may be shown, from a paper by Dr. Alan Robertson (1952), that the expected variance between means of line crosses is

$$F\sigma_G^2 + F^2\sigma_D^2$$

where F is Wright's coefficient of inbreeding,

σ_G^2 is the additive component of variance,

and σ_D^2 is the variance due to dominance.

In the present experiment, the value of F when the lines were crossed was 0.5. The expression then reduces to

$$\tfrac{1}{2}\sigma_G^2 + \tfrac{1}{4}\sigma_D^2$$

It can also be shown, from the same paper, that the term $F\sigma_G^2$ represents the component of variation due to the general combining ability of the lines, while $F^2\sigma_G^2$ is a component ascribable to special combining ability of lines in particular crosses. It has been shown that, for this particular stock, the within family heritability of litter size is of the order of 0.15 (Falconer, 1956). Now,

$$h^2_w = \frac{\frac{1}{2}\sigma_G^2}{\sigma_P^2}$$

where h^2_w is the within full-sister group heritability, and σ_P^2 is the within family phenotypic variance in litter size, which is about 5. Substituting,

$$0.15 = \frac{\frac{1}{2}\sigma^2_G}{5}$$

from which $\frac{1}{2}\sigma^2_G \sim 0.75$

This value of 0.75 is therefore the expected one for the variation due to general combining ability of the lines. The estimate will be divided between the variance components between dam lines and between sire lines, each of which therefore has the expectation of 0.375. It might also be divided between two generations. As the error variance in a character of low heritability is expected to be large, it would be optimistic indeed to hope for a significant value for the variation in general combining ability, without an experiment on a much larger scale.

By similar reasoning, the variation in special combining ability is also expected to be low. In the first place, only in special circumstances will the dominance variance be much greater than the additive component (see, for instance, Mather, 1949). Secondly, only quarter of this variation will be represented as variation in special combining ability.

When the actual estimates are derived it will be seen that these apprehensions are fully justified. Nevertheless, in a field that is largely unknown, any estimate is of potential value. The analysis will be presented in some detail, as the method whereby the estimates are obtained is of some intrinsic interest.

The data have to be analysed in two distinct classifications. The first of these concerns crosses (irrespective of whether the cross is AB or BA), reciprocal members of the same cross, and error variance. In the second classification, the sums of squares are partitioned between dam lines, sire lines and interaction. We shall consider the two classifications in this order.

The error variance is of course common to both. For the purpose of estimating error variance, replicate crosses were required. A replicate cross involved the same two lines, the parent of the same sex always coming from the same line. In the first cross, JR_3, the error variance was estimated from 22 duplicate sets, and in the second

cross from 40 triplicate sets. These were the figures obtained:-

	Degrees of Freedom	Error Mean Square
JR_3	22	8.41
JRX	80	3.18

In both generations, it proved to be immaterial whether the replicates were taken from the same litters or from different litters of a line or cross. In both cases, the mean square was larger when the replicates involved the same litters, though not significantly so.

Apart from replicates, reciprocal crosses were also arranged to test for maternal effects. The reciprocal mean square was tested against the replicate mean square. Reciprocals are of course different members of the same cross; the variance between crosses is therefore tested against the reciprocal mean square - against the variance between members of the same cross.

The data is summarised in tabular form. All the analysis was done within the three major groups that constituted the experimental population. "Group" refers to a set of lines of common origin, within which all crossing was done.

TABLE IIIa

ANALYSIS OF CROSSES - GENERATION JR_3

Inbred parents - crossbred offspring

	D.F.	Sums of Squares	Mean Square	Significance Test
TOTAL	106	760.617		
BETWEEN GROUPS	2	38.916	19.46	$F^2_{104} = 2.80$
WITHIN GROUPS	104	721.701	6.94	$P > .05 < .10$
BETWEEN CROSSES	44	311.441	7.08	$F^{44}_{38} = 1.19$
WITHIN CROSSES	60	410.260	6.84	$P > .20$
BETWEEN RECIPROCALS	38	225.260	5.93	$F^{38}_{22} = 0.70$
BETWEEN REPLICATES	22	185.000	8.41	$P(\text{of } \frac{1}{F}) \sim .20$

TABLE IIIa.

ANALYSIS OF CROSSES - GENERATION JRX

Crossbred parents - crossbred young

	D.F.	Sums of Squares	Mean Square	Significance Test
TOTAL	146	734.707		
BETWEEN GROUPS	2	85.395	42.70	$F^{2}_{144} = 9.47$ $P < .001$
WITHIN GROUPS	144	649.319	4.51	
BETWEEN CROSSES	38	224.742	5.91	$F^{38}_{26} = 0.90$ $P > .50$
WITHIN CROSSES	106	424.570	4.01	
BETWEEN RECIPROCALS	26	169.903	6.54	$F^{26}_{80} = 2.05$ $P \sim .01$
BETWEEN REPLICATES	80	254.667	3.18	

There are no detectable differences between crosses in either generation. This indicates that the selection of good crosses out of the array of possible ones could not be effectively made. It also suggests that neither general nor special combining abilities are exerting any marked influences on the variance between crosses.

In the second crossbred generation, there is a significant difference between reciprocals, indicating the influence of maternal effects on litter size. This finding is less surprising than the absence of such a difference in first crossbred generation. It will be recalled that at the end of the inbreeding stage, the difference between lines was significant.

The large value for the error mean square in the JR3 generation is a disconcerting feature of the data. It suggests strongly that for accurate determinations of variance due to the general and special combining abilities, large scale experimentation would be required. Even in the second cross, the error variance is much larger than the expected value of any component of variance.

We now come to the second method of analysing the variance in crosses. From this classification we shall estimate the variation in the general combining ability of the lines, and special combining ability in crosses. The former is the sum of the

components of variance between lines as female parents and between lines as male parents. The latter is the interaction component of variance, the corresponding degrees of freedom and sums of squares being obtained by subtraction in the usual manner. The appropriate analysis of variance tables for the two generations are as follows.

TABLE IVa

BETWEEN LINE ANALYSIS OF VARIANCE - JR_3

	D.F.	S.S.	M.S.
TOTAL (Within groups)	104	721.701	
ERROR (Between replicates)	22	185.000	8.41
BETWEEN DAM-LINES	22	132.267	6.01
BETWEEN SIRE-LINES	23	175.135	7.61
INTERACTION	37	229.299	6.20

TABLE IVb

BETWEEN LINE ANALYSIS OF VARIANCE - JRX

	D.F.	S.S.	M.S.	
TOTAL (Within groups)	144	649.312		
ERROR (Between replicates)	80	254.667	3.18	
BETWEEN DAM-LINES	22	164.921	7.50	$F^{22}_{80} = 2.36$; $P < .01$
BETWEEN SIRE-LINES	22	223.117	10.14	$F^{22}_{80} = 3.19$; $P < .001$
INTERACTION	20	6.607	0.33	$F^{20}_{80} = 0.10$; $P(\text{of } \frac{1}{F}) < .001$

In the first crossbred generation, the biggest mean square of all is the error variance, and obviously nothing is significant. In the second cross, there are significant differences between the lines when used both as female and as male parents. In other words, the fertility of a crossbred dam is affected by the genotype of both her parents. The between sire-line mean square is greater than the between dam-line mean square, but the difference is not significant. The interaction mean square, when tested against error, is highly significantly too small. In order to explain this, we must examine the constitution of the mean squares. When this is done, all normal tests

of significance are seen to be of an indicative value only.

The first requirement is to determine the constitution of the sums of squares. This proved to be awkward on account of the non-orthogonal system used for crossing the lines. I am indebted to Dr. Alan Robertson for showing how the desired information could be extricated from the data. Dr. Robertson points out that the principle of the analysis is that given by Henderson (1953).

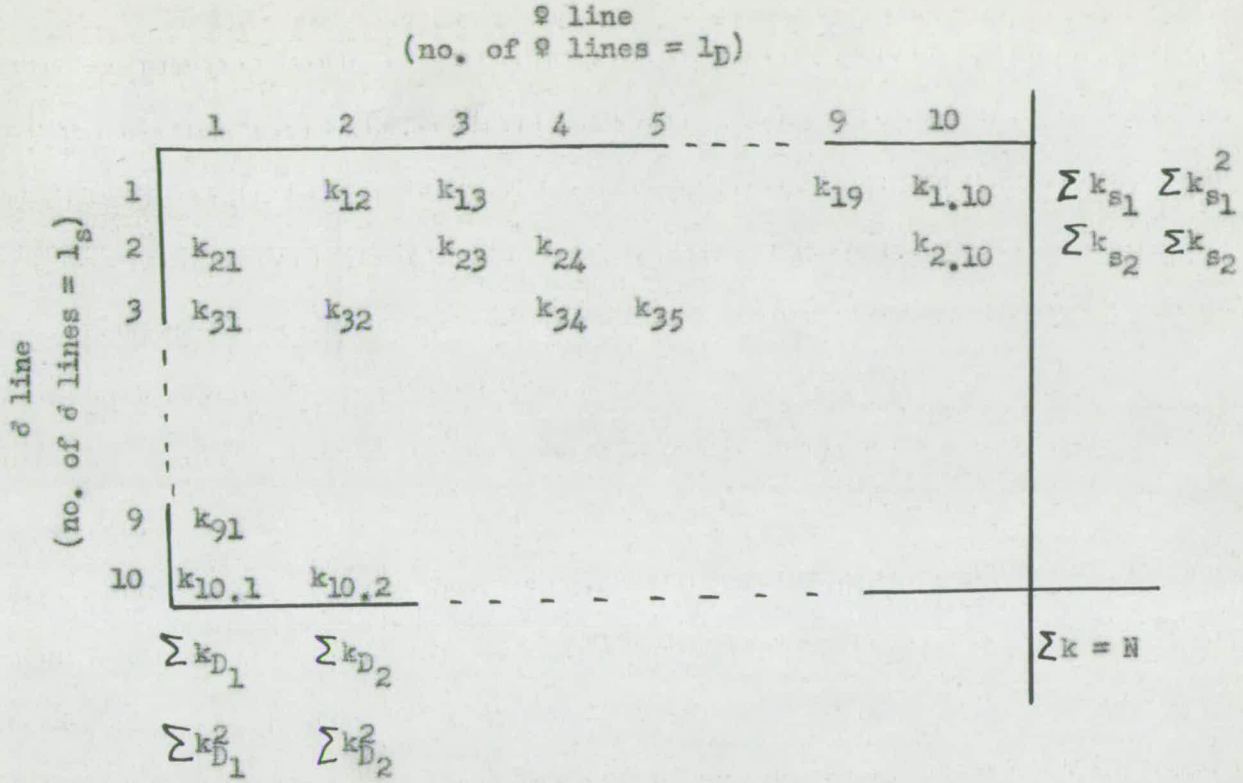
Excluding algebraic terms for the moment, let us consider what constitutes the sums of squares. A sire-line was mated at random to four dam-lines, giving a certain mean performance for that sire-line. Now, assuming for present purposes that there are differences between dam-lines, had that particular sire-line been mated to a different set of dam-lines, its mean performance would have a slightly different expectation. What this means in effect is that the between sire-line mean square contains some of the between dam component. By the same reasoning, the between dam mean square contains a between sire component. In other words, if we are to determine the between dam and between sire components from the raw data, both will be overestimated.

This has repercussions on the interaction sum of squares. This figure is obtained by subtraction, and as the between sire and between dam figures are magnified, the interaction sum of squares is underestimated.

There are therefore three unknowns, σ_s^2, the between sire line component of variance, σ_d^2, the between dam line components, and σ_I^2, the interaction component. The error variance, σ_E^2, can be measured directly, as it contains no other source of variation.

There are three equations from which the three unknowns can be estimated. The coefficients to be used for weighting the unknowns in the equations are an extension of the correction, k_o, given by Snedecor (1946) for unequal subsample numbers. They are calculated in the following manner.

The system of crossing, described previously, was of this general design:-



k refers to the number of observations per cell, either one or two in the JR_3 generation, and mostly ones and threes in JRX. The total number of observations in a group of 10 lines is N. Calculate for each column and row k and k^2. This enables us to write down the expected coefficients in the sums of squared observations (uncorrected sums of squares), conveniently expressed in tabular form:-

		EXPECTED COEFFICIENTS OF			
		σ_ϵ^2	σ_I^2	σ_D^2	σ_S^2
Total	T	N	N	N	N
Sire lines	S	l_s	$\sum \frac{\Sigma k_s^2}{\Sigma k_s}$	$\sum \frac{\Sigma k_s^2}{\Sigma k_s}$	N
Dam lines	D	l_D	$\sum \frac{\Sigma k_D^2}{\Sigma k_D}$	N	$\sum \frac{\Sigma k_D^2}{\Sigma k_D}$
Correction	C	1	$\frac{\Sigma k^2}{N}$	$\frac{\Sigma(\Sigma k_D)^2}{N}$	$\frac{\Sigma(\Sigma k_s)^2}{N}$

We now prepare another table, and by subtracting the correction term, we derive the appropriate coefficients of components in the sums of squared deviations from the mean.

		EXPECTED COEFFICIENTS OF σ^2_E	σ^2_I	σ^2_D	σ^2_S
Total	T-C	N-1	$N-\frac{\Sigma k^2}{N}$		
Between sire lines	S-C	l_s-1			
Between dam lines	D-C	l_D-1	etc.	etc.	etc.
Interaction + Error	T-S-D+C	$N-l_s-l_d+1$			

The last row gives the coefficients for the various components of variance in the combined sums of squares for interaction plus error. It is obtained by subtracting the between sire and between dam coefficients from the total.

The tables have to be calculated separately for the three major groups. The final operation is simply to summate the coefficients for the components in the sums of squares for three groups. These were the figures obtained for the first crossbred generation, JR_3.

	EXPECTED COEFFICIENTS OF σ^2_E	σ^2_I	σ^2_D	σ^2_S	Observed Sums of Squares
Total	104	102.769	93.323	93.440	721.701
Between sire lines	23	30.770	21.324	93.440	175.135
Between dam lines	22	29.837	93.323	20.508	132.267
Interaction + error	59	42.162	-21.324	-20.508	414.299

The first column is of course the degrees of freedom corresponding to the source of variation listed - the number of times it contains the error variance. The "interaction plus error" contains a known amount of error.

	D.F.	S.S.	
Interaction + Error	59	414.299	
Error	22	185.000	
Interaction	37	229.299	, by subtraction

We are now in a position to write down the equations from which we can determine the three unknowns for the first crossbred generation, JR_3.

$$30.770\ \sigma_I^2 + 21.324\ \sigma_D^2 + 93.440\ \sigma_S^2 = -18.272$$
$$29.837\ \sigma_I^2 + 93.323\ \sigma_D^2 + 20.508\ \sigma_S^2 = -42.868$$
$$42.162\ \sigma_I^2 - 21.324\ \sigma_D^2 - 20.508\ \sigma_S^2 = -81.834$$

The right hand side of the equation is obtained by subtracting from the sum of squares the number of times it contains the error variance, e.g. for the first equation, the value is 175.135 - (23 x 8.41) = - 18.272 etc.

The solution of these simultaneous equations is

$$\sigma_S^2 = +0.376$$
$$\sigma_D^2 = +0.017$$
$$\sigma_I^2 = -1.748$$

The value for σ_I^2 is negative. As a component of variance cannot take a real negative value, our best estimate of the interaction component is zero. This leaves us with two equations to estimate two unknowns.

$$21.324\ \sigma_D^2 + 93.440\ \sigma_S^2 = -18.272$$
$$\text{and } 93.323\ \sigma_D^2 + 20.508\ \sigma_S^2 = -42.868$$

which give

$$\sigma_S^2 = -0.316$$

$$\text{and } \sigma_D^2 = +0.528$$

σ_S^2 is now negative. By the same procedure as before

$$93.323\ \sigma_D^2 = -42.868$$
$$\text{which gives } \sigma_D^2 = -0.459$$

The conclusion is that of the three components of variance in the first crossbred generation all are about zero, though large sampling errors preclude precise estimation. As suggested earlier, this is not entirely unexpected.

For the second crossbred generation, JRX, the coefficients of the components of

variance in the sums of squared deviations were obtained in exactly the same manner as before, with the following results.

	EXPECTED COEFFICIENTS OF				Observed
	σ^2_E	σ^2_I	σ^2_D	σ^2_S	Sums of Squares
Total	144	139.106	127.104	125.210	649.312
Between sire lines	22	51.462	39.460	125.210	223.117
Between dam lines	22	55.446	127.104	41.550	164.921
Interaction + Error	100	32.198	-39.460	-41.550	261.274
Error	80				254.667
Interaction	20				6.607

From which we derive the three equations:-

$$51.462\,\sigma^2_I + 39.460\,\sigma^2_D + 125.210\,\sigma^2_S = 153.091$$
$$55.446\,\sigma^2_I + 127.104\,\sigma^2_D + 41.550\,\sigma^2_S = 94.895$$
$$32.198\,\sigma^2_I - 39.460\,\sigma^2_D - 41.550\,\sigma^2_S = -57.053$$

The solution is

$$\sigma^2_S = +1.080$$
$$\sigma^2_D = +0.364$$
$$\sigma^2_I = +0.068$$
$$\text{c.f. } \sigma^2_E = +3.183$$

The interaction mean square is very small indeed, and if these figures are reliable, then special combining ability was practically non-existent in this particular situation. It certainly seems to have a very much smaller effect than the general combining ability of the lines.

D. SUBSIDIARY OBSERVATIONS

1. BODY WEIGHT IN RELATION TO INBREEDING AND CROSSING

This study takes note of the weight of females only, at a standard age of six weeks. By this time mice are normally sexually mature and can be mated. The weight of the males was not considered, as only some were retained after weaning at three weeks. Weight was considered primarily because of the known strong effect of mother's six week weight on the size of her litter. In view of this, it was considered that during the inbreeding and crossing study of litter size, it would be interesting to follow the

response in another character genetically correlated with it.

The six week weight of females used for breeding was kept for the following generations; the inbreeding coefficient corresponding to that generation is also shown.

Generation	JR_0	JR_1	JR_2	JR_3	JRX
Inbreeding coefficient	0	.25	.375	.50	0

In addition to females kept for breeding, all other females were weighed in the last two generations, JR_3 and JRX, as they were retained in any case for other purposes. The data for these generations are therefore considerably augmented by these extra records.

The last generation, JRX, refers to the weights of crossbred females reared by inbred dams. It would have been desirable to obtain a further generation mean - that of outbred females by crossbred dams. Such mice were born, measuring the fertility of crossbred mothers, but the collection of the corresponding weight data would involve retaining a large number of mice for six weeks for no other purpose. This did not seem justifiable.

The generation means, in grammes, are shown in the following table. A graphical representation is given in Figure 11.

TABLE V

SIX WEEK BODY WEIGHTS OF FEMALES - GENERATION MEANS

Generation	JR_0	JR_1	JR_2	JR_3	JRX
Inbreeding coefficient	0	.25	.375	.50	0
Group H	22.6	21.2	20.0	21.9	22.4
L	22.2	21.6	20.1	21.1	21.3
C	18.8	19.7	20.1	21.4	20.3
Unweighted mean of 3 groups	21.2	20.8	20.1	21.5	21.3
litter size	8.12	6.73	5.82	5.69	6.20

For the first two generations, there was an indication of a slight decline in body weight. This seemed likely, as the increase in weight in lines 21 to 30 was not

taken very seriously. This was because these mice, when they were first obtained, suffered from an infestation of mites which certainly affected the six week weight of the JR_0 generation. They were then decontaminated, and an improvement in body weight was expected as a result of this treatment. However, all three groups show an increase in body weight during the last generation of inbreeding, but no further improvement on crossing.

The general impression then is that the character has not responded in any way to the effects of inbreeding and crossing. At this point, however, reference must be made to the influence of maternal effects discussed earlier. As litter size declines, body weight should tend to increase through the consequent advantage in milk supply. Since we know that body weight as a character declines on inbreeding, it appears that in this particular case the depression has been more or less balanced by the advantage gained through the simultaneous reduction in litter size. Likewise, when the lines were crossed, the potential increase in body weight was nullified by the increase in litter size.

The components of variance within and between lines are given below in tabular form. They are illustrated in Figure 12, from which it is seen that the fluctuations from generation to generation are so large that no trend of any kind is evident. As far as we are able to judge, the data in this respect resemble those of litter size.

TABLE VIa

BODY WEIGHT - WITHIN LINE COMPONENT OF VARIANCE

Generation	JR_0	JR_1	JR_2	JR_3
Inbreeding coefficient	0	.25	.375	.50
Group H	3.54	2.36	1.72	7.78
L	2.20	6.59	1.87	3.46
C	3.40	3.83	1.86	4.50
Unweighted mean	3.05	4.26	1.82	5.25

TABLE VIb

BODY WEIGHT - BETWEEN LINE COMPONENT OF VARIANCE

Generation	JR_o	JR_1	JR_2	JR_3
Group H	1.77	3.21	9.52	1.56
L	1.09	2.36	4.75	2.57
C	7.01	4.40	6.76	4.58
Unweighted mean	3.29	3.32	7.01	2.90

For the sake of completeness, the analysis of variance in body weight is presented here in summary form. The analysis was carried out in the manner previously described for litter size. To simplify the arithmetic, the measurement of a cross was taken to be the mean weight of females in a litter, irrespective of the number on which it was based. Statistical refinements were not considered to be worthwhile.

In the case of body weight, there is only one generation to analyse, - crossbreds reared by inbred mothers. 15 duplicate litters were available for estimating error.

These are the two analyses of variance tables corresponding to the ones used for litter size:-

TABLE VII

ANALYSIS OF CROSSES - BODY WEIGHT

	D.F.	Sum of Squares	Mean Square	Significance test
TOTAL	82	393.483		
BETWEEN GROUPS	2	45.494	22.75	$F^{2}_{80} = 5.23$ $P < .001$
WITHIN GROUPS	80	347.989	4.35	
BETWEEN CROSSES	53	215.519	4.07	$F^{53}_{12} = 0.66$ $P(\text{of } \frac{1}{F}) > .10$
WITHIN CROSSES	27	132.470	4.91	
BETWEEN RECIPROCALS	12	74.045	6.17	$F^{12}_{15} = 1.58$ $P \sim .20$
BETWEEN DUPLICATES	15	58.425	3.89	

TABLE VIII

BETWEEN LINE ANALYSIS OF VARIANCE IN BODY WEIGHT

	D.F.	Sum of Squares	Mean Square	Significance Test
TOTAL (Within groups)	80	347.989		
Error (Between duplicates)	15	58.425	3.89	
BETWEEN DAM LINES	22	115.200	5.24	$F_{15}^{22} = 1.35$ $P > .20$
BETWEEN SIRE LINES	22	90.303	4.10	$F_{15}^{22} = 1.05$ $P \sim .50$
INTERACTION	21	84.061	4.00	$F_{15}^{21} = 1.03$ $P \sim .50$

With the exception of the difference between the three major groups, none of the tests are significant.

The coefficients of components in sums of squared deviations from the mean were calculated as before.

	EXPECTED COEFFICIENTS OF				Observed
	σ_E^2	σ_I^2	σ_D^2	σ_S^2	Sums of Squares
Total	80	78.92	71.64	71.59	347.989
Between sire lines	22	28.64	21.36	71.59	90.303
Between dam lines	22	28.53	71.64	21.20	115.200
Interaction + Error	36	21.75	-21.36	-21.20	142.486
Error	15				58.425
Interaction	21				84.061

The three equations for estimating the components of variance are

$$28.64\,\sigma_I^2 + 21.36\,\sigma_D^2 + 71.59\,\sigma_S^2 = 4.613$$
$$28.53\,\sigma_I^2 + 71.64\,\sigma_D^2 + 21.20\,\sigma_S^2 = 29.510$$
$$21.75\,\sigma_I^2 - 21.36\,\sigma_D^2 - 21.20\,\sigma_S^2 = 2.266$$

from which

$$\sigma_S^2 = -0.156$$
$$\sigma_D^2 = +0.344$$
$$\sigma_I^2 = +0.292$$
$$\text{cf. } \sigma_E^2 = +3.895$$

Equating $\sigma_S^2 = 0$

$$28.53\,\sigma_I^2 + 71.64\,\sigma_D^2 = 29.510$$
$$21.75\,\sigma_I^2 - 21.36\,\sigma_D^2 = 2.266$$

whence

$$\sigma_I^2 = 0.365$$
$$\text{and } \sigma_D^2 = 0.265$$

The order of magnitude of these components again indicates that for accurate estimation, the scale of the experiment is inadequate. If one were to venture a comment, the excess of the between dam component over the between sire suggests the presence of strong maternal effects on body weight. The apparent existence of interaction variance points to the possibility that certain combinations of lines may yield superior crosses for body weight, even though there is little differentiation between the lines themselves.

2. MORTALITY OBSERVED AT BIRTH

Mice found dead when the litter is first recorded are either stillbirths or neonatal deaths. As mice regularly dispose of dead young, there is only a partial recovery in the records of deaths due to these two causes. Nevertheless, the data are presented here as they seemed to be indicative of the relative importance of the effect of inbreeding in the litter and in the dam on the loss of young. This loss may occur either during late pregnancy or during the critical period at or immediately following parturition.

The character was measured as the percentage live young of the total found when the litter was recorded. The standard error of the binomial was calculated. Figure 13 shows the percentage found alive at birth plotted with two standard errors, and was constructed from the following figures:-

TABLE IX

PERCENTAGE MICE FOUND ALIVE

Generation	Alive	Dead	Total	p	σ_p
J0	846	30	876	.966	.0061
JR_o	887	60	947	.937	.0079
JR_1	528	42	570	.926	.0109
JR_2	500	33	533	.938	.0104
JR_3	666	19	685	.972	.0063
JRX	1247	22	1269	.983	.0036

Two differences are seen from Figure 13 to be statistically significant, the first between J0 and JR_o, and the second between JR_2 and JR_3. Both these correspond to changes in the heterozygosity in the litter. No other differences approach significance.

It would appear therefore that with regard to late foetal or neonatal viability, or to both, the effect of inbreeding and crossing on the litter is markedly greater than the same effects on the mother.

3. WEANING RATE

The measurement of this character was taken as the percentage mice weaned of those found alive at birth. The standard error of the binomial was calculated. Figure 14 shows a plot of the percentage reared per generation, together with two standard errors. These were the figures.

TABLE X

PERCENTAGE MICE REARED

Generation	Weaned	Not weaned	Total	p	σ_p^2
J0	815	31	846	.963	.0065
JR_o	780	107	887	.879	.0109
JR_1	458	70	528	.867	.0149
JR_2	446	54	500	.892	.0139
JR_3	608	58	666	.913	.0109
JRX	1147	100	1247	.920	.0077

The first point worthy of note in the Figure is that the crossbred young with crossbred parents fall below the level of the original outbreds. But when the outbreds were examined for the same measurement in the nearest contemporaneous generation, it was found that there had been a general decline, and that the difference between crossbreds and outbreds was no longer significant.

Secondly, the percentage weaned dropped significantly immediately inbreeding in the young commenced.

Thirdly, the improvement on crossing is difficult to estimate as the differences are not statistically significant, and also in view of the improvement that accompanied the last generation of inbreeding. But taking the data as they stand, there was greater improvement in the first than in the second cross. This agrees with Wright's (1922b) observation on guinea-pigs.

IVB. RESULTS

THE EFFECT OF INBREEDING AND CROSSING ON COMPONENTS OF FERTILITY

This subsidiary experiment was calculated to determine how various components of fertility react individually to the effects of inbreeding and crossing. The measurements taken were (i) corpora lutea counts - to estimate the ovulation rate (ii) implantation rate (iii) live embryos at 16 days. These data were collected for five groups of matings:-

I	Inbred A ♀	x	Inbred A ♂
II	Inbred A ♀	x	Inbred B ♂
III	Inbred ♀	x	Crossbred ♂
IV	Crossbred ♀	x	Inbred ♂
V	Crossbred ♀	x	Crossbred ♂

A fuller account of the experimental design has been given in an earlier section.

The group means for the three measurements were as follows. The same figures are illustrated graphically in Figure 15.

TABLE XI

GROUP MEANS - COMPONENTS OF FERTILITY

	GROUP	CORP. LUT.	IMPLANTS	LIVE EMBRYOS
I	Inbred A ♀ x Inbred A ♂	10.037	8.444	7.111
II	Inbred A ♀ x Inbred B ♂	9.862	7.552	6.690
III	Inbred ♀ x Crossbred ♂	10.200	8.800	7.767
IV	Crossbred ♀ x Inbred ♂	9.929	8.857	8.071
V	Crossbred ♀ x Crossbred ♂	10.267	9.100	7.833

Looking at Figure 15, the general impression is that the five groups do not differ greatly in their ovulation rate, but that by implantation they have fanned out. No further differentiation appears among the groups between implantation and late pregnancy.

There is one anomaly in these results. The mean, both of implants and of live embryos, is lower for inbred parents with crossbred young than for inbred parents with inbred young. This differs from expectation, and indeed from the behaviour of the

corresponding groups in the main experiment, just described. But when the difference was tested, it was found to be not significant; in fact, when the analysis of variance of group means was carried out, none of the differences were significant for any of the three measurements. The data were grouped where possible, with the same result. Whenever a test of significance was done, the within group variance was always too large to establish real differences. In view of the practical work involved, this was disappointing.

However, at the level of empirical observation, there appears to be less variation between groups in mean number of corpora lutea than in either of the other two measurements. With respect to corpora lutea there are, of course, only two groups; Groups I, II and III constitute the inbred female group, while Groups IV and V are crossbred females. There was a difference in mean number of corpora lutea of 0.068 in favour of the crossbred group. The standard error of the difference was 0.33, indicating that the difference between the two means is totally insignificant. The figures were obtained with 142 degrees of freedom. As the fiducial limit, we take twice the standard error of the difference. We can therefore state with confidence that if crossbred females have a higher ovulation rate than inbreds, then the mean difference is no greater than 0.66 of an egg.

Falconer (unpublished) found the same result with mice of the same stock. He had two groups only - inbred parents with crossbred young and crossbred parents with crossbred young. He had larger number per group, and the difference in mean number of implants was significant at the five per cent level; the two groups had shown no difference in ovulation rate.

It seems reasonably clear therefore that the difference in fertility of inbred and outbred mice is not in their ovulation rate, but rather in the rate of implantation. Whether this is due to failure of fertilisation or to mortality of blastocysts is a matter of conjecture. It is a point that can be determined fairly easily by the microscopic

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examination of eggs a few hours after copulation.

One or two "byproducts" of this study may be mentioned. The negative correlation of -0.41 between right and left sides of a mouse in number of corpora lutea was quoted in Chapter 2. However, by implantation, evidence of this right-left asymmetry had disappeared. No significant correlation between sides was found either in number of implants or in number of embryos.

The reason for this is not hard to find. It means that crowding within an uterine horn affects the loss. Thus a mouse with an extreme distribution between sides will contribute largely to the negative covariance in corpora lutea counts. But the horn with the larger number of eggs suffers a greater loss. This will lead to a reduction in the negative correlation between sides. These were the figures obtained, pooling the data from all groups:-

TABLE XII

LOSS OF EGGS IN RELATION TO NUMBER PER SIDE

C.L. per horn	Total horns	Total C.L.	Total losses	Fraction lost = p
1	1	1	0	.0000
2	19	38	4	.1053
3	31	93	18	.1935
4	61	244	35	.1434
5	65	325	45	.1385
6	42	252	40	.1587
7	38	266	40	.1504
8	21	168	31	.1845
9	4	36	12	.3333
10	2	20	6	.3000

The χ^2 for a linear trend is highly significant, showing that the greater the ovulation rate, the greater the percentage loss. This is the full χ^2 analysis.

	D.F.	χ^2	Probability level
TREND	1	11.285	$< .001$
RESIDUAL	8	4.539	$\sim .80$
TOTAL	9	15.824	$< .10 > .05$

The residual χ^2 is not in the least significant, confirming the deduction that hetero-

geneity between groups is due to a definite linear trend. The distribution of the loss shows no deviation from the expected, but even by excluding the last two groups, where the loss seems to be greatest, the trend χ^2_1 is still 6.031, with a probability of less than 0.02.

If the analysis is done within mice, as distinct from within uterine horns, the trend disappears. This is to be expected from the negative correlation of corpora lutea between sides.

In conclusion, we can say that although the scale of this subsidiary experiment was inadequate to assess the effect of inbreeding and crossing on all the three components of fertility that were examined, the decline in litter size on inbreeding is not due in any measure to a decrease in the ovulation rate of the mother. To this extent, the subsidiary work fulfilled its purpose.

V. DISCUSSION

The interpretation of the experimental data has been rendered somewhat imprecise by the complexity of the character of litter size. The difficulties involved can be attributed in no small measure to the dual genetic determination of the character, and the relative contributions of the dam and of the litter itself are seldom clearly distinguishable. In addition, we have strong maternal effects on litter size, and their interplay with inbreeding depression adds further intricacies. Superimposed on all this are manifold environmental influences that act with different force in different circumstances. The examination of the underlying genetic situation will therefore be severely limited in its scope until such time as the constituent factors of litter size are more perfectly understood. In the meantime, though we may be restricted to the empirical exploration of the field, all information is of potential value.

To some extent, we have seen the contributions to litter size of the dam and of the litter itself acting separately. At the commencement of inbreeding, reduced viability of the unborn litter had a marked effect which was not fully reflected in increased litter size when the inbred lines were crossed. This contradiction has not been adequately resolved. It may, of course, be a spurious chance effect, though this is unlikely, as Eaton (1953) noted the same result in a similar situation. It would be interesting to see whether the same phenomenon would appear if the work were to be repeated. If the effect is a real one, then it reflects our inadequate understanding of the subject, and any prediction of the effects of inbreeding on litter size in the mouse becomes a hazardous affair.

When the lines are crossed, the effect of the crossbreeding of the dam appears to be greater than the effect of the crossbreeding of the litter. This also presents a contradiction, for when the effect of the inbreeding of the dam was first introduced in the second generation of inbreeding, its impression was not in the least perceptible, as the decline was no greater than what would be expected from the further inbreeding of the litter.

When these complementary phenomena are considered together, the possibility that they may be due to sampling errors becomes reduced. It would seem that the decline in litter size is not linearly related to inbreeding when its effect in the litter and the dam are considered separately. There may also be some kind of interaction between the two. Until we have further knowledge of the processes involved, we can only deal with the situation empirically.

As body weight did not alter materially during the experiment, we can postulate that the maternal effects on litter size have been buffered to a large extent by the maternal effect on body weight. In order to see where these maternal effects conceivably exert their influence, it is simpler to consider what happens when inbreeding is first introduced, that is, to outbred parents with inbred young. Reduced viability of the unborn young give a smaller litter size at birth. As the milk supply of the mother is thus far unimpaired, these young will be larger at six weeks, and will themselves bear larger litters. Simultaneously, if six week weight declines on inbreeding, there will be a similar maternal effect on litter size but acting in the opposite direction. As it happened, inbreeding decline and a simultaneous positive maternal effect kept six week weight at a fairly constant level, so that neither of the two maternal effects on litter size would be transmitted. Litter size in the next generation was therefore determined by inbreeding effects on the dam and further inbreeding in the litter, without the complications arising from maternal effects.

When litter size was examined as separate components, it was found that the first of them, ovulation rate, remained unaffected by inbreeding. The superiority of crossbreds over inbreds appears to be due to a difference in the rate of implantation. More experimental evidence is required to show whether this is due to lack of fertilisation or to preimplantational loss of blastocysts.

The component of fertility affected by inbreeding appears to be different in mice and pigs. Squires, Dickerson and Mayer (1952), and King and Young (1956) both found

that ovulation rate declines on inbreeding. The reason for the discrepancy is not clear. The genetic control of the character of litter size is possibly rather different in the two animals, and caution must be exercised when extrapolating from one to the other.

The question of major interest in this work was the comparison of the crossbred population with the original outbreds. The two were indistinguishable in litter size and body weight. Though this is what theoretical considerations lead us to expect, the suggestion is often encountered that crossbreds have some intrinsic merit. From the results of the experiment described here, unselected crossbreds are in no way superior to outbreds. Inbred lines are in effect samples of the gametes of the original outbred population. A cross between two lines thus reconstitutes one individual from the original outbreds. When the lines are fully inbred, this individual can be replicated at will.

In this light, inbreeding and crossing appear to have no intrinsic benefit of their own other than to afford rapid and effective means of selection. Genotypes with a poor performance are eliminated, and if some lines are discarded before crossing, the crossbred population will be composed by the union of superior gametes and should therefore show a higher mean performance, with respect to any trait on which selection acted. Selection between lines is undoubtedly effective in the case of litter size in mice. Reference was made in Chapter 2 to the JU inbred stock, in which inbreeding was carried out without artificial selection. Natural selection soon reduced the ten original lines to three; these three showed little if any decline in litter size on inbreeding. Yet when they were crossed, the crosses showed strong hybrid vigour (Falconer, unpublished), illustrating the effectiveness of between line selection. Simultaneously, within line selection in a parallel stock (JS) proved to be totally ineffective in arresting the decline in litter size on inbreeding.

Equally ineffective, from the results described in the last chapter, would be selection between crosses of inbred lines. Neither the first nor the second crossbred generation revealed any evidence of variation between the crosses. If inbreeding and crossing is to be used as a method of improving litter size in the mouse, it thus seems clear that there will be no improvement without selection, and that the only selection likely to be effective is that between inbred lines.

Lack of variation between crosses points to the absence of specific combining ability and indeed to the absence of variation in general combining ability. Yet, how is the latter to be reconciled with the effectiveness of selection between lines? One way would be to postulate a negative specific combining ability, where a cross between two high lines would give an average performance, and likewise low by low. This seems absurd. In any case, if it were so, the interaction component of variance could hardly be so minute. A more likely explanation lies in the use of partly inbred material, where only half the additive and a quarter of the dominance variance was expressed. This explanation is obliquely supported by Dr. Falconer's experience with the JU and JS material. Only when the inbreeding coefficient was raised above 0.5 did natural selection begin to act between lines. The conclusion is that if inbreeding and crossing is to be used as a method of improving a character such as litter size in the mouse, then the improver must be prepared if need be to carry his inbreeding somewhere beyond the level of 0.5. Natural selection will then probably do his work for him, if he is prepared to foot the bill.

From our data, can we infer anything about the underlying genetic situation, always bearing in mind the reservations made earlier when discussing the complexity of the character? We can say with some confidence that specific combining ability, and thus the dominance variance, appear to be relatively unimportant. This would hardly be the case if the dominance variance were very much greater than the additive variance. Such a situation would exist if overdominant loci, with genes at intermediate frequencies, were

contributing largely to the total variance. Employing a somewhat subjective assessment, we can say that the idea of overdominance at a number of loci is not supported. This of course is an application of the law of parsimony, and is in no way an established scientific truth.

There is one other reason why overdominance is probably not of very great importance. It will be recalled that the good lines from Dr. Falconer's JU stock did not show inbreeding depression. This would not be expected if litter size in the mouse was controlled by many overdominant loci.

The application of these results will be limited to situations of similar genetic control, but in final conclusion, what are we to advocate as a method of improving a character such as litter size in the mouse? If inbreeding and crossing is to be employed, then the tedium and cost of maintaining lines at a fairly advanced state of inbreeding must be endured. Even then, many crosses may not be successful in increasing litter size.

In view of this, it is welcome news that the within-family selection experiment, carried out on the same stock in this laboratory, has by now produced a sizeable difference between the high and low lines (Falconer, 1956 and unpublished). We have just stated that there is no reason to suppose that the character is controlled by many overdominant loci, which would preclude the successful outcome of a selection programme. Only in such circumstances would inbreeding and crossing be a better method of improving the character.

With mammals, at least, the maxim is an obvious one, though it may not always be universally applied. It is that the most troublesome and most costly method of selection should be used only as a last resort, where other methods do not produce the desired result.

VI. SUMMARY

Theoretical considerations of the phenomenon of hybrid vigour indicate that the mean of crosses between unselected inbred lines should coincide with the mean of the original outbreds from which the inbreds were derived. Experimental evidence on this point is lacking, and the work described here was primarily calculated to test it.

Three groups of mice of slightly different origin provided the basic material for thirty inbred lines, which were perpetuated through three generations of full-sib mating. At this stage, the lines were crossed at random within the groups, as further inbreeding would probably lead to loss of lines through infertility. The main character studied was the size of the first litter; six week weight of the females was also recorded.

The mean litter size of the crossbreds agrees well with theoretical expectation. It is argued that any improvement following inbreeding and crossing would be due to selection against bad genotypes during inbreeding. The system as such has no intrinsic merit other than affording means of effective selection which might not otherwise be possible.

A crossbred is regarded as a reconstituted individual from the outbred population. Any desired individual can be replicated at will by crossing the same two lines.

Decline in litter size on inbreeding is marked. There is some recovery when the lines are crossed (inbred mothers with crossbred young), followed by a much greater increase when the crossbreds are used as parents.

The decline in litter size when the litter is first inbred (still with outbred parents) is greater than the recovery when the inbred lines are crossed. Similarly, the increase caused by crossbreeding in the parents is not matched by a corresponding decline when the effect of inbreeding in the parents is first introduced.

Litter size is markedly affected by the genotype of the dam and of the litter itself. This complicates the interpretation of the experimental data. Maternal effects on litter size, and their interplay with inbreeding depression, are also discussed.

A method is described whereby variance components relating to general and specific combining abilities may be determined. As anticipated, these are very small.

Body weight showed no inbreeding depression. This result does not agree with previous work, and it is postulated that the potential decline has been counteracted by a maternal effect resulting from the reduction in litter size.

It is suggested that if inbreeding and crossing is to be used as a method of improving a character such as litter size in the mouse, then the improver must be prepared if need be to work with a more advanced level of inbreeding than that employed in this experiment. The obvious conclusion from this is that other methods of selection should be employed where possible.

ACKNOWLEDGEMENTS

I express my gratitude to Dr. D. S. Falconer for his care and supervision, to Professor C. H. Waddington for laboratory facilities, and to Dr. A. Robertson and Dr. B. Woolf for statistical advice.

I am indebted to Dr. H. P. Donald for leave of absence from the Animal Breeding Research Organisation, and to the Agricultural Research Council for supporting me throughout the course of this work.

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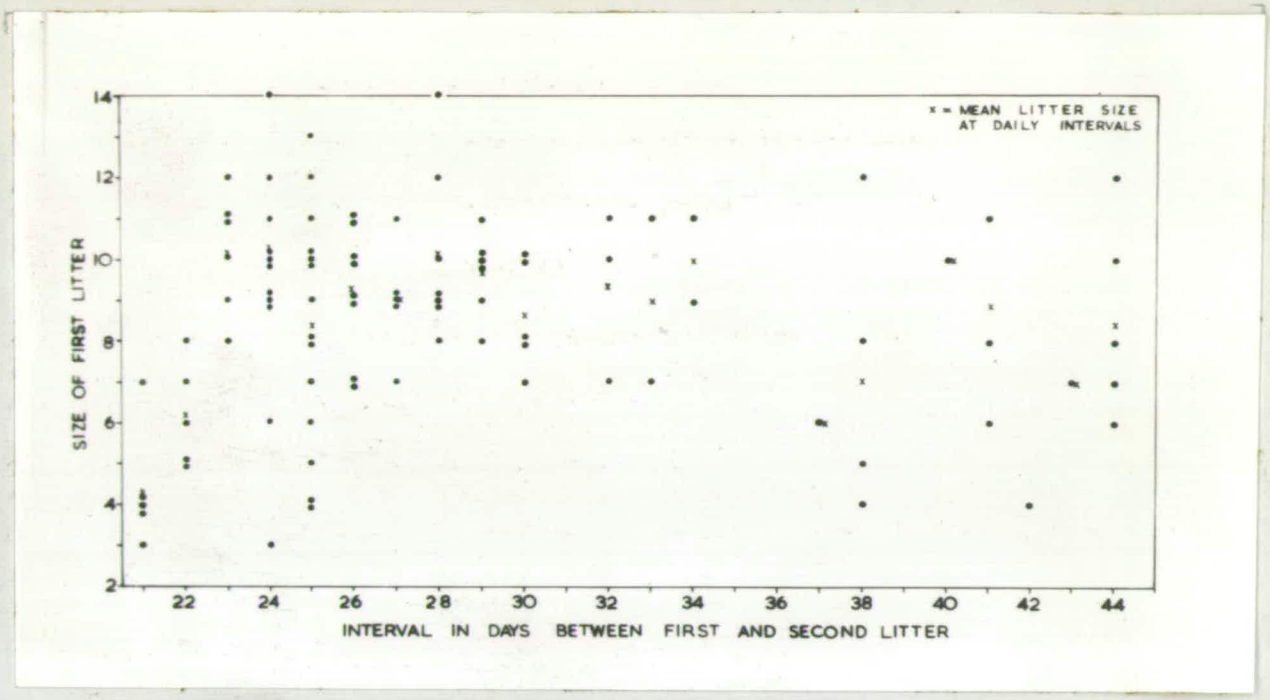

Fig. 1. Relationship between size of first litter and the interval between first and second litters, in an unselected outbred stock.

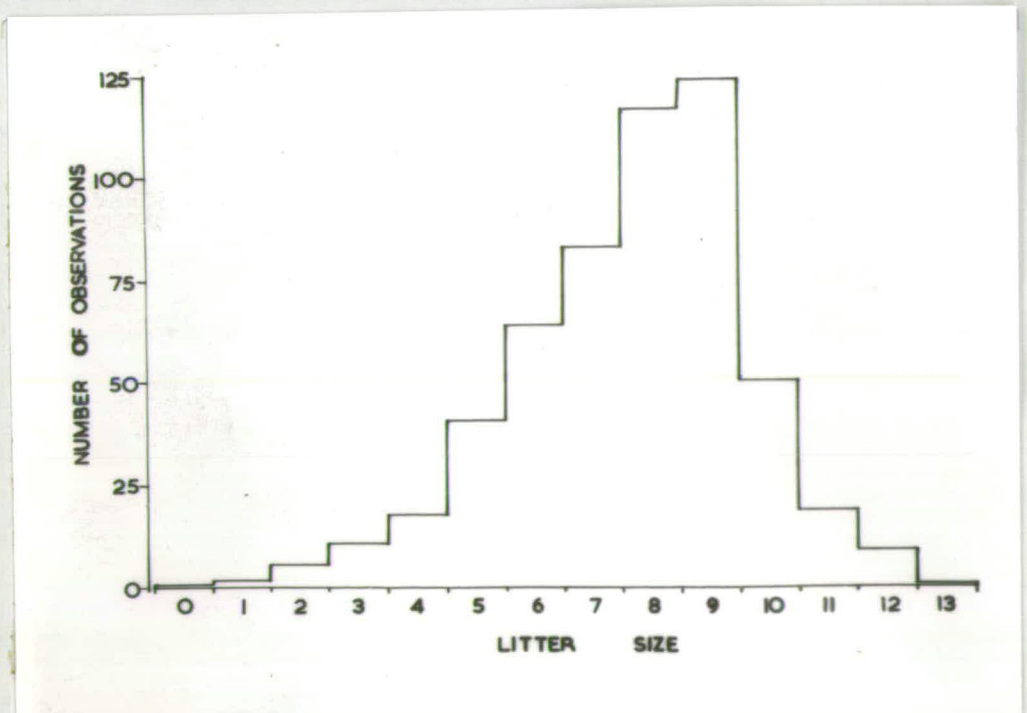

Fig. 2. Distribution of litter size.

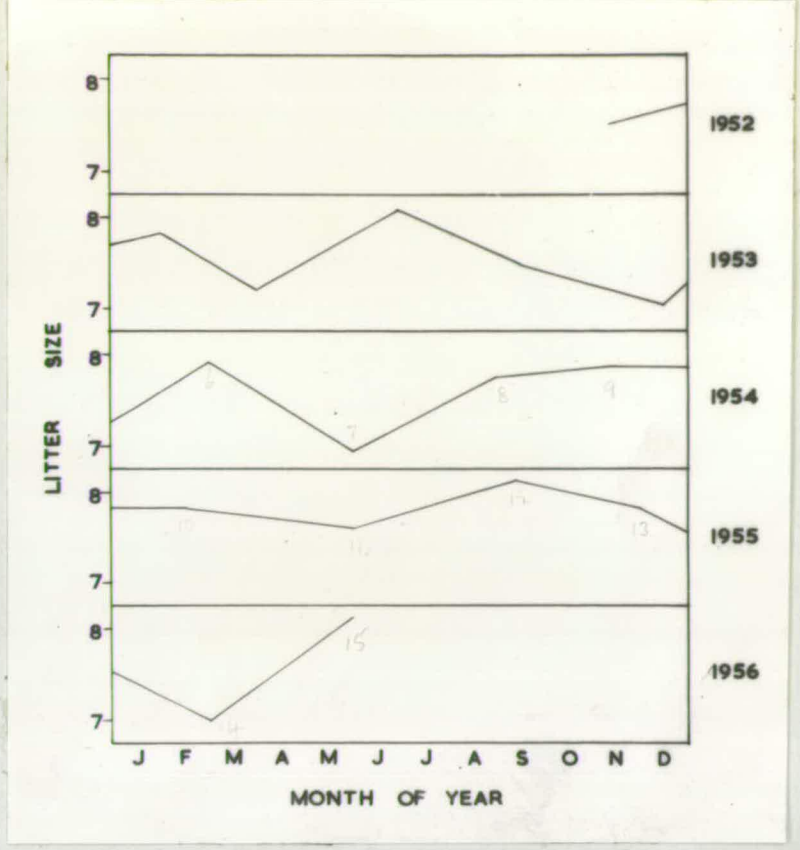

Fig. 3. Seasonal variation in litter size in an unselected outbred stock. Generation means plotted against month of year in which the particular generation was born.

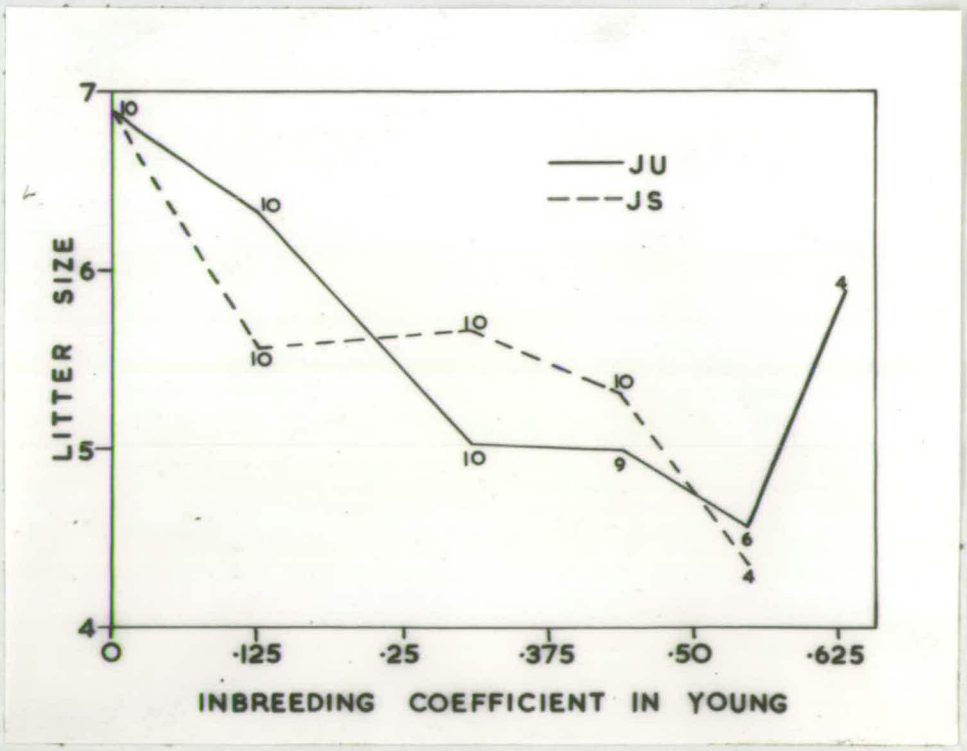

Fig. 4. Effect of inbreeding on mean litter size. Generation means plotted against inbreeding coefficient in the litter. JU - inbreeding without selection; JS - inbreeding with selection against small litters.

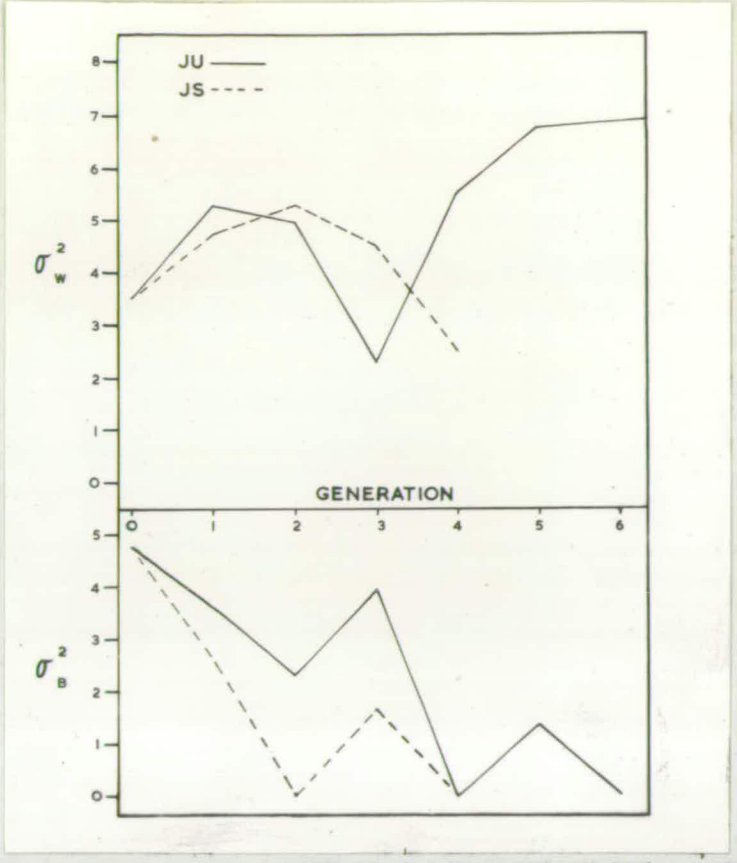

Fig. 5. Within and between line components of variance in litter size in two inbred stocks, JU and JS. σ^2_W - component of variance within lines; σ^2_B - component of variance between lines.

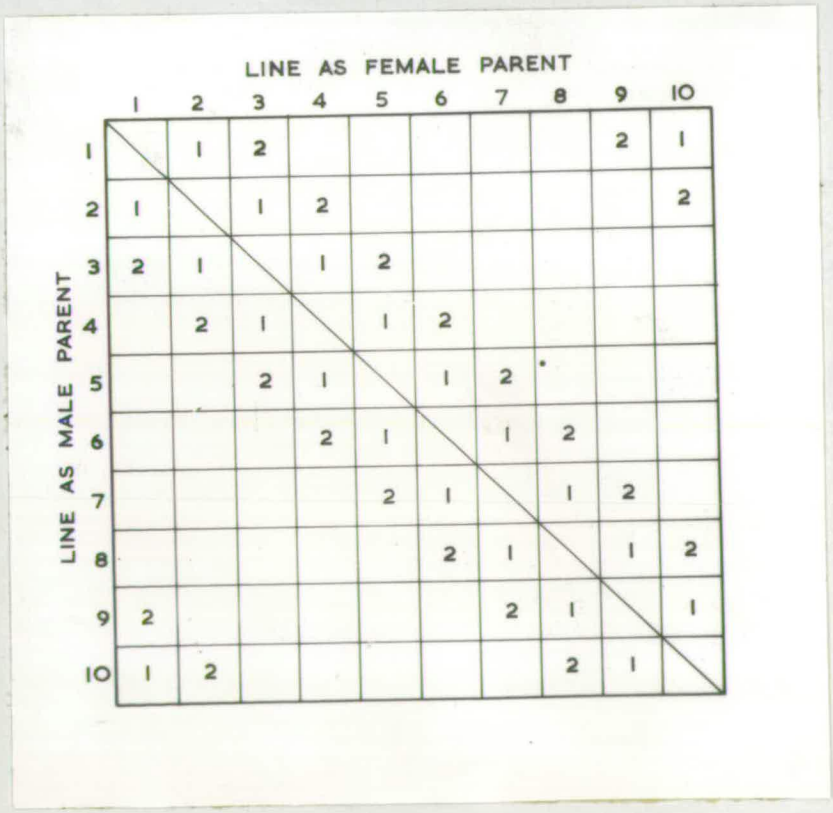

Fig. 6. The principle of the scheme of crossing the inbred lines. The number in each cell represents the number of matings of that type.

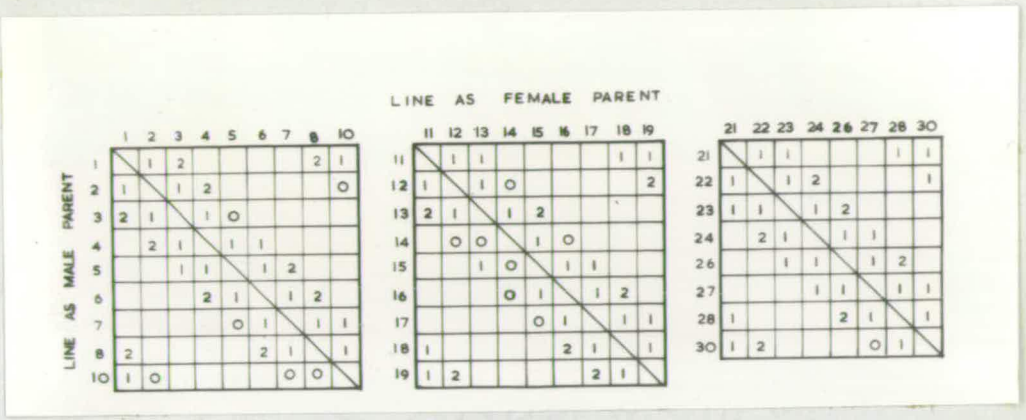

Fig. 7. Number of matings employed in the first crossbred (JR_3) generation, of the type shown.

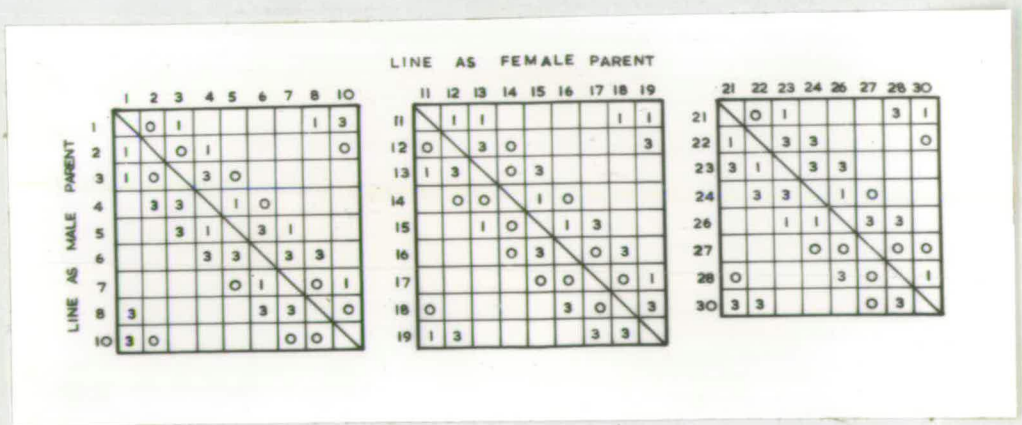

Fig. 8. Number of matings employed in the second crossbred (JRX) generation, of the type shown.

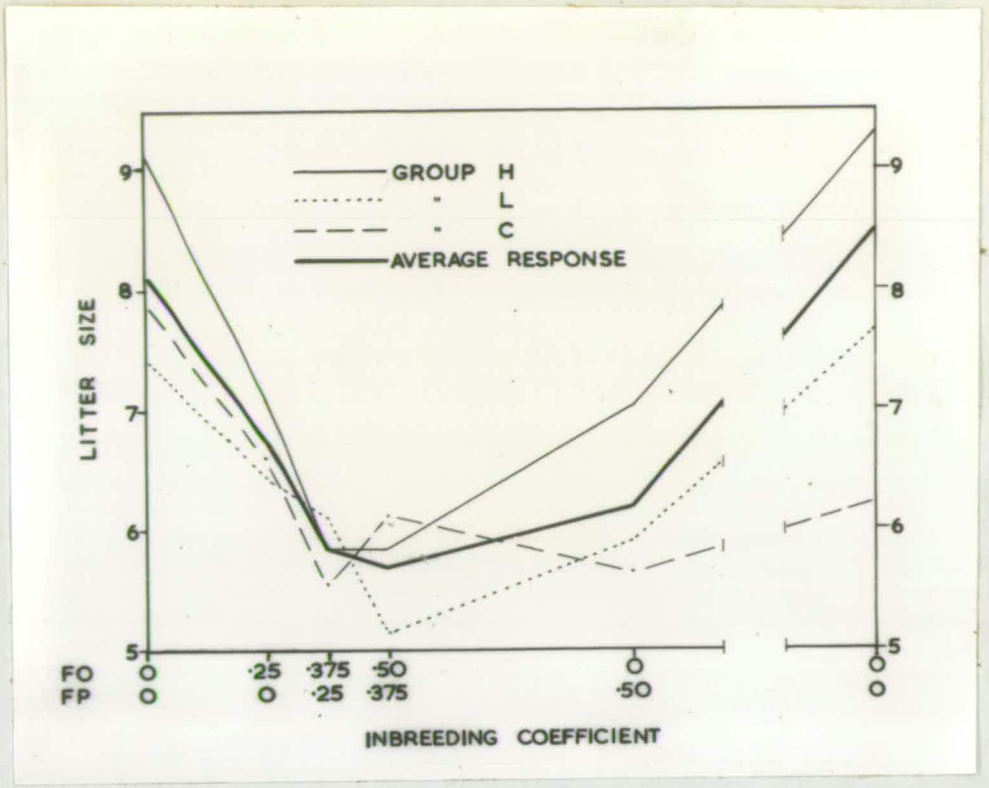

Fig. 9. Response of litter size to the effects of inbreeding and crossing. Mean litter size plotted against the coefficient of inbreeding. F.O. - coefficient of inbreeding in offspring; F.P. - coefficient of inbreeding in parents.

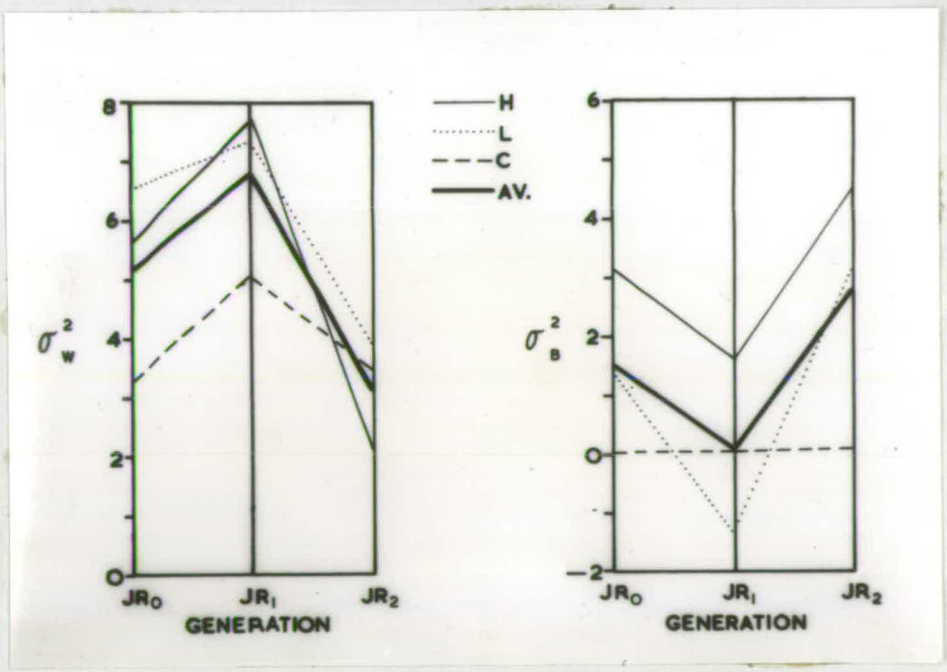

Fig. 10. Within and between line components of variance in litter size during the inbreeding phase of the experiment. σ^2_w - component of variance within lines; σ^2_B - component of variance between lines.

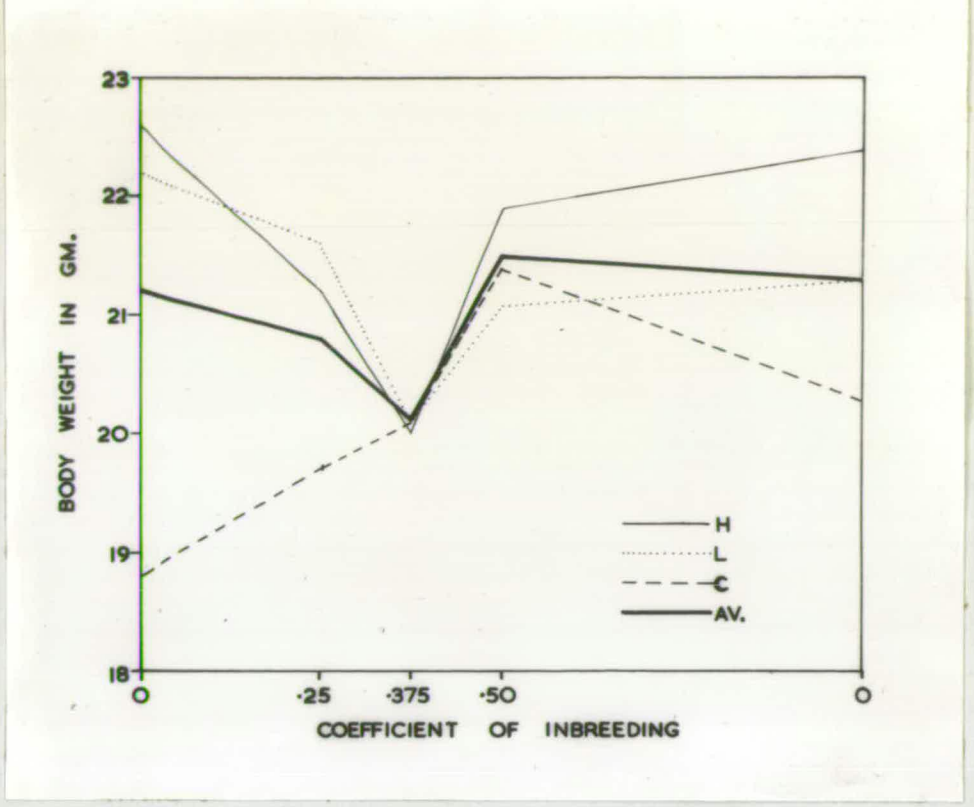

Fig. 11. Response of body weight to the effects of inbreeding and crossing. Mean six-week weight of females plotted against coefficient of inbreeding.

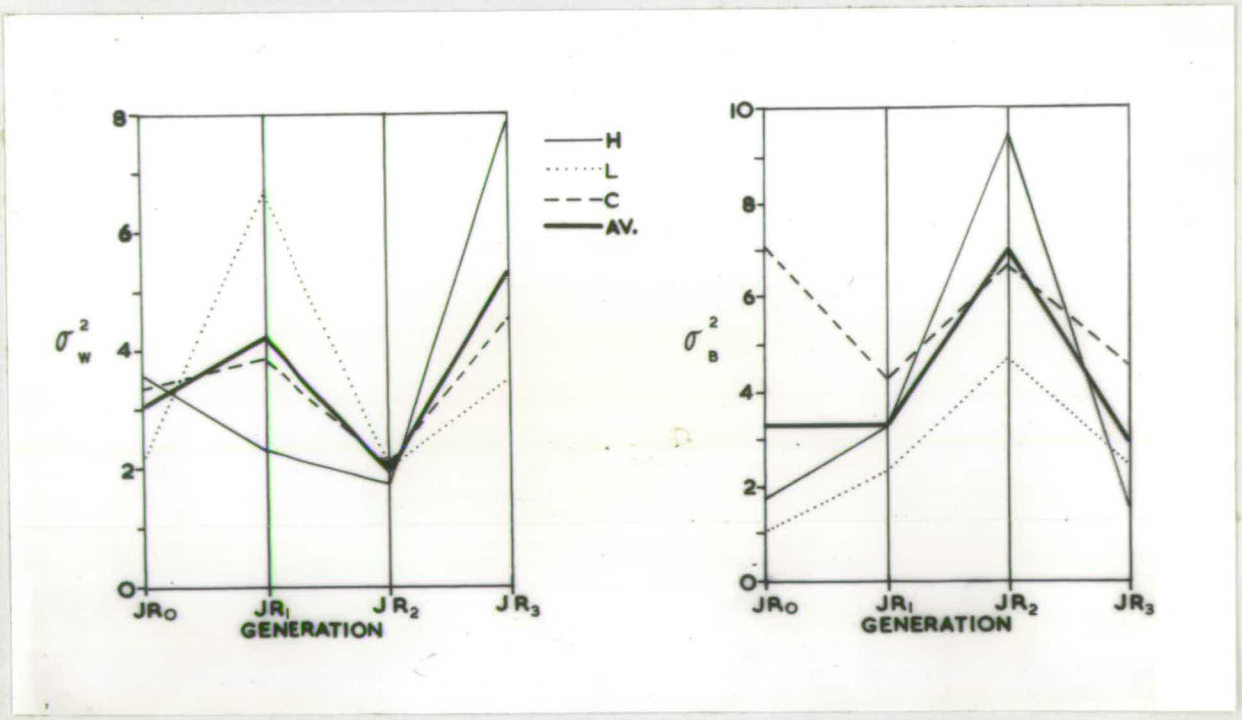

Fig. 12. Within and between line components of variance in body weight during the inbreeding phase of the experiment. σ^2_W - component of variance within lines; σ^2_B - component of variance between lines.

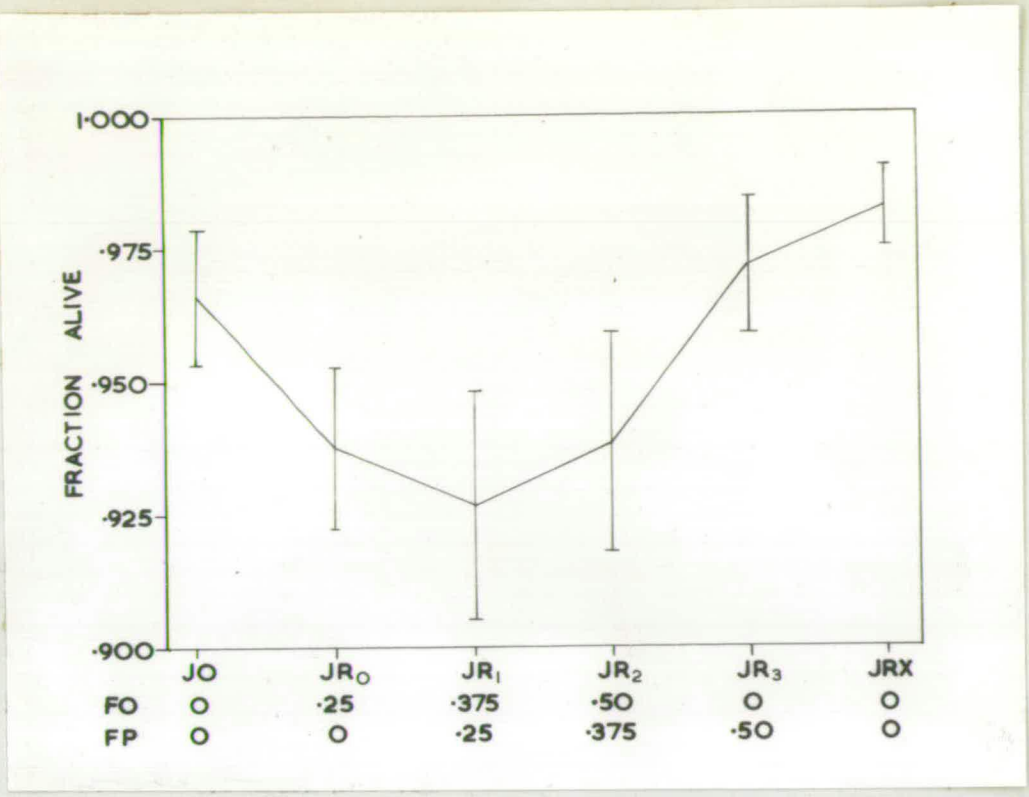

Fig. 13. Fraction of mice found alive at birth, plotted with two standard errors each side of the mean, against coefficient of inbreeding in offspring (F.O.), and in the parents (F.P.)

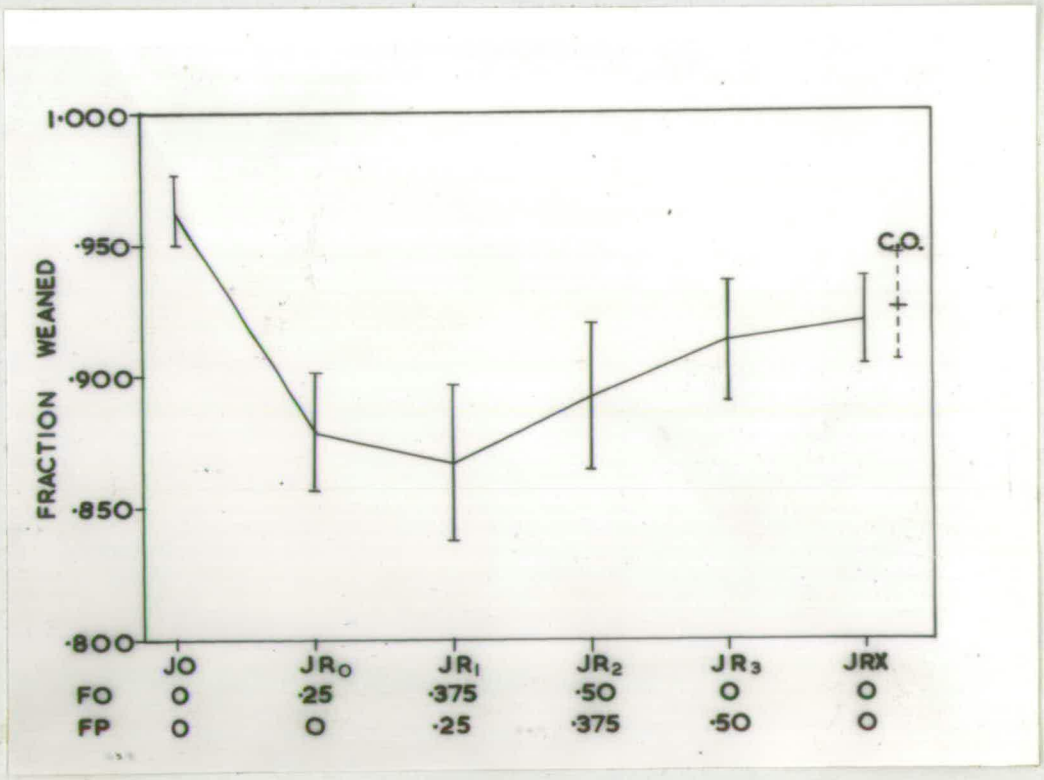

Fig. 14. Fraction of mice weaned of those born alive, plotted with two standard errors each side of the mean, against coefficient of inbreeding in offspring (F.O.), and in the parents (F.P.). C.O. = contemporaneous outbred.

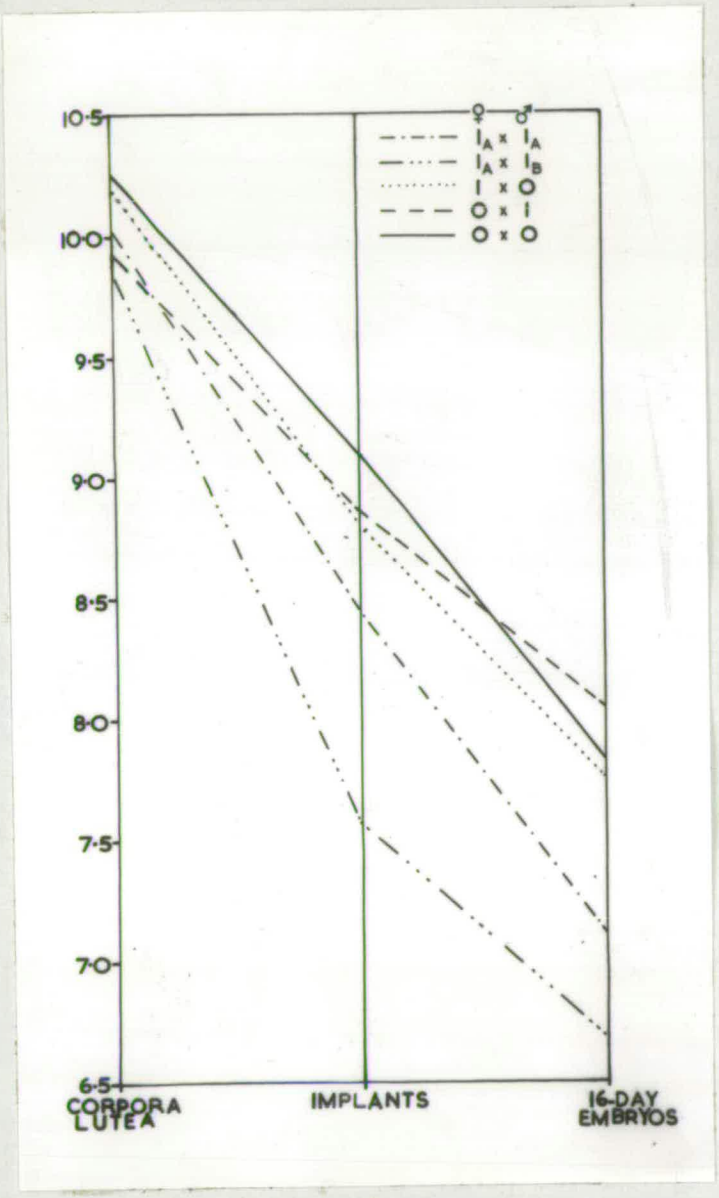

Fig. 15. Mean number of corpora lutea, implants, and live embryos at 16 days, in five groups of mice.
I_A x I_A - Inbred female, inbred young; I_A x I_B - Inbred female, crossbred young
I x O - Inbred female x crossbred male; O x I - Crossbred female x inbred male
O x O - Crossbred female x crossbred male.